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# CONTENTS OF PREVIOUS NUMBER

## SEPTEMBER 1938. NUMBER 3


Giant Follicular Lymphoblastomyopathy With or Without Splenomegaly; Its Transformation into Polymorphous Cell Sarcoma of the Lymph Follicles and Its Association with Gaucher's Disease, Lymphatic Leukemia and an Apparently Unique Disease of the Lymph Nodes and Spleen—A Disease Entity Believed Heretofore Undescribed. Douglas Symmers, M.D., New York.

Histology of the Bone Marrow in Aplastic Anemia. C. P. Rhoads, M.D., and D. K. Miller, M.D., New York.

Incidence of Mild Degrees of Atrophy in the Fasciculus Gracilis. Donald Duncan, Ph.D., Galveston, Texas.

Syncytioid Proliferation of the Reticuloendothelial System (Reticulendotheliosis): Report of a Case and Comments on the Literature. Milton B. Sachs, M.D., Baltimore.

Adaptation of Human Polymorphonuclear Leukocytes by Tuberculoprotein. William B. Wartman, M.D., Cleveland.

Historical Review of Metachromasia: Contribution to Pathology. B. H. [illegible], M.D., New York.

Case Reports:
Primary Fibromyxoma of the Heart with Sarcomatous Extensions. C. P. Larson, M.D., and [illegible] A. Sheppard, B.Sc., Fort Stellacoom, Wash.
Syphilis of Frontal Skull Before 1250. Henry Stephen Lippincott, M.D., Glendale, Ariz.

Laboratory Methods and Technical Notes:
A Combination Toluidine-Methyl Green-Pyronine Stain for Formaldehyde-Fixed Tissue. James R. Lisa, M.D., New York.

Notes and News.

Abstracts from Current Literature.

Society Transactions:
New England Pathological Society.
New York Pathological Society.
Chicago Pathological Society.

Book Reviews.

Books Received.

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VOLUME 26 OCTOBER 1938 NUMBER 4

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# CEREBRAL LESIONS IN HYPOGLYCEMIA

## II. SOME POSSIBILITIES OF IRREVOCABLE DAMAGE FROM INSULIN SHOCK

A. B. BAKER, M.D.

MINNEAPOLIS

With the increasing use of artificial hypoglycemia in the treatment of schizophrenia, acne, morphinism and other conditions, the problem of the cerebral changes that might result from such therapy becomes paramount. The extensiveness and variability of the hypoglycemic symptoms indicate that severe cerebral alterations of a temporary or even permanent nature must occur, but for some reason the report on such cerebral changes are few. As a rule the symptoms in hypoglycemia, regardless of their severity, if the patient is treated early, appear to be transitory. However, in the more severe and prolonged attacks the lesions may be more profound and persist for days or become permanent. There appears to be a need for careful studies of the neurologic lesions produced by hypoglycemia, particularly those that lead to permanent disability or death.

In a previous publication, Lufkin and I [1] reported the cerebral alterations in 3 cases of hypoglycemia. The studies in those cases were augmented by experiments on animals and by a review of the literature. Many investigators had reported damage to the nerve cells as a most constant finding. Our studies at that time revealed that the alterations of the nerve cells in the cases studied were of no apparent pathologic significance. It appeared that the most common pathologic observation was that of numerous new and old hemorrhages scattered irregularly throughout the brain tissue.

Since these earlier investigations, 2 new cases of hypoglycemia have been studied. In each case changes in the brain were so extensive that an immediate report seems indicated, especially in view of the bearing that these studies may have on the use of severe hypoglycemia as a therapeutic agent. The microscopic lesions in both brains were very similar and hence will be described together.

From the Department of Medicine of the Division of Nervous and Mental Diseases of the University of Minnesota.

1. Baker, A. B., and Lufkin, N. H.: Arch. Path. **23**:190, 1937.

REPORT OF CASES

CASE 1.—A white man aged 64 years was apparently well until December 1934, when, during a routine examination, glycosuria was found. He was placed on a modified diet, about which he was careless. In August 1935 he began to experience polydipsia and polyuria. In December of that same year painless jaundice appeared. His local physician again prescribed a diet and insulin. The jaundice cleared up, but edema of the lower extremities appeared. He was first seen by me in May 1936, at which time he was acutely ill. Since he had glycosuria (4 plus), an attempt was made to regulate his diet and control the diabetes. Great difficulty was encountered at once because the patient's insulin requirements seemed to fluctuate from day to day. After three weeks of hospitalization he suddenly presented an insulin reaction, with dry skin, deep breathing and loss of consciousness; the blood sugar was too low to measure. He was given sugar and responded fairly well but had two more hypoglycemic attacks within the next twenty-four hours. His tolerance to sugar continued to vary, as evidenced in frequent and often severe hypoglycemic attacks. After much variations in his diet and dosage of insulin, he was finally regulated and discharged from the hospital in March 1937, after a stay of ten months.

He was observed from time to time and seemed to be progressing fairly well until October 1937, when he was rushed to the hospital in a semistuporous condition. Three days prior to this admission he had contracted an infection of the upper part of the respiratory tract, and his wife had given him the usual amount of insulin but used U-80 instead of U-40. He passed into a state of hypoglycemia. Under treatment at the hospital he seemed to respond for a few hours. Then he suddenly showed mild spasticity of the right side. He sank into a fairly deep stupor, and convulsive movements developed in the right extremities, with a deviation of the head and eyes to the right. His left pupil became irregular and sluggish. The course was rapidly downhill. The right-sided involvement progressed to complete paralysis. In spite of the maintenance of a normal level of blood sugar, the patient failed to respond and died without regaining consciousness after a terminal illness of eight days.

A complete postmortem examination was made. There were no significant somatic changes except in the pancreas. This organ weighed 45 Gm. and was firm and nodular. Sections showed no islands. There were chronic fibrosis and inflammation of an extreme degree with a few abscesses.

External inspection of the brain showed nothing abnormal. Coronal sections revealed petechiae scattered throughout the cerebral hemispheres. These were most marked in the temporoparietal and frontal regions. The hemorrhages were diffuse and showed no tendency to group formation. An occasional extravasation was large, measuring almost 1 mm. in diameter.

In the region of the left inferior parietal lobule, just lateral to the lenticular nucleus there was a region of softening and partial cavitation, measuring 2 by 1 cm. This cyst was multilocular and was surrounded by an area of softened brain substance.

CASE 2.—A white man aged 60 years was first seen in July 1935, at which time he complained of dyspnea, nervousness, excessive perspiration and peculiar attacks that had been present for five months. These spells started in February 1935 with periodic weakness and dizziness. A few weeks later he began to have periods of unconsciousness accompanied by abnormal activities. At one time he sat up in bed, screamed, thrashed about and was very hard to manage. During another

spell he became foolish, sitting down and playing in the dirt like a child. The spell prior to his admission occurred after he had been picking berries all day. He suddenly took the money with which he was to pay the help, threw it on the ground and became noisy. He was taken home, where he grabbed a milk pail and started chasing the cows. When his neighbors tried to pacify him, he became intensely angry. His more severe attacks lasted about one to three hours, and recovery was spontaneous. On regaining consciousness he complained of extreme weakness and was unable to recall any of the events occurring during or immediately preceding the seizure.

On admission it was discovered that the patient's thyroid gland was enlarged. This, together with the history of weakness, nervousness and a basal metabolic rate of +33 per cent, suggested a diagnosis of toxic goiter. The goiter was removed on July 9. Following his discharge from the hospital, the patient continued to feel weak and dizzy. About every other week he experienced mild spells without complete loss of consciousness. These were characterized by profuse perspiration, drowsiness and mental confusion. Recovery was spontaneous after about two hours, but if he ate candy recovery was hastened.

In January 1936 he suffered a severe seizure, occurring just before his breakfast. He began thrashing about the room, striking the walls and breaking the furniture. His local physician gave him an intravenous injection of dextrose, following which he immediately recovered. He was hospitalized. Examination again gave negative results except for the blood sugar, which varied from 38 to 73 mg. per hundred cubic centimeters of blood. Lowering of the blood sugar by fasting produced apprehensiveness, marked perspiration, irritability and short periods of amnesia. These symptoms were at once relieved by sugar. At this time it became apparent that the patient was suffering from recurring hypoglycemia, and an attempt was made to regulate his diet in order to avoid the seizures. This proved unsuccessful, so in July 1936 a laparotomy was performed and the pancreas explored. No tumor was found. Following this operation, the patient continued to have frequent severe seizures, during which he would become so violent that his family was afraid to live with him. During some of the later attacks, he would often be unconscious for a period of days. Usually during these severe prolonged periods of unconsciousness he was brought to the hospital for treatment.

His final admission occurred in June 1937, at which time he was comatose and frothing from the mouth. The blood sugar was 18 mg. per hundred cubic centimeters, and the temperature was 102 F. He was given large quantities of dextrose, and although the blood sugar rose to a normal level, the patient did not improve. He began to have right-sided tonic convulsions, which continued for hours. His deep reflexes became hyperactive and irregular. The eyegrounds were normal. The blood sugar was kept at 100 mg. per hundred cubic centimeters, but the patient continued in a coma for eight days and died without responding to any type of therapy.

Permission for a complete autopsy was obtained. The lungs revealed slight diffuse emphysema and very mild edema. Present bilaterally were a few small areas of early pneumonic consolidation. The abdominal viscera were apparently normal with the exception of the pancreas, which contained in the upper portion of its head an adenoma, measuring 1.5 by 1.5 by 1 cm. The tumor was well circumscribed except for its lower border, which merged with the surrounding pancreas. This adneoma was composed almost entirely of island tissue in anastomosing cords and irregular sheets. The composition of the tumor was the same throughout. The rest of the pancreas, its ducts and the common bile ducts were uninvolved.

The brain, after it had received an injection and been fixed in solution of formaldehyde U. S. P., presented a curious picture. Large areas of the hemispheres remained soft. These softened areas were most extensive in the region of the right superior parietal and marginal gyri. No external hemorrhages were observed. The basal vessels revealed only a slight amount of patchy sclerosis.

Coronal sections were made through the hemispheres. A well circumscribed, walled-off cavity, 2 by 1.5 by 4 cm., was found in the uppermost portion of the left anterior central gyrus. Its inner lining was soft and granular. The surrounding brain tissue was filled with numerous petechiae that tended to localize within the cortex. Within the right parieto-occipital region there were two large cavities, beginning in the vicinity of the angular gyrus and extending posteriorly for about 3 cm. (fig. 1). The uppermost was well demarcated and extended almost to the medial surface of the brain; it tapered off posteriorly and merged into the

Fig. 1.—Large cavitations observed in case 2. Note their irregular outline and their localization to the white substance of the brain.

occipital tissue. The lower cyst was situated more deeply. It measured 3 by 5 by 5 cm., was irregular in outline and contained soft necrotic nonhemorrhagic walls. It extended posteriorly almost to the occipital pole and was, with the exception of one small area, sharply limited to the white substance. Much of the posterior half of the left cerebral hemisphere was replaced by large cavities, which were situated primarily in the white substance but in certain areas extended through the cortex to the surface of the brain. Their walls were irregular and surrounded by areas of softened tissue.

Numerous petechiae were observed throughout the frontal and occipital regions. These hemorrhages seemed to be situated primarily in the cortex and were often clumped but not destructive.

*Microscopic Observations in Cases 1 and 2.*—Routine study of the nervous system included sections taken from numerous regions throughout the cortex and

white substance and from the basal ganglions, the midbrain, the cerebellum and the medulla. The sections, even when examined superficially, were impressive because of the widespread, almost diffuse nature of the cerebral damage. In fact, microscopically, normal fields were decidedly fewer and smaller than abnormal regions.

Vascular Changes: There was congestion of the blood vessels. Numerous hemorrhages, which varied in magnitude and destructiveness, were scattered irregularly throughout the brain tissue (fig. 2 *A*). The petechiae were usually small and tended to localize within the cortex and around the areas of cavitation. Most of the bleedings were perivascular, and those in which a vessel was not visible certainly suggested a vascular origin by their uniform rounded arrangement. Many of the petechiae were grouped but did not fuse to form larger hemorrhages. The brain tissue around them was not softened and did not appear to be undergoing degenerative changes. Many of the hemorrhages, however, were definitely destructive. They were larger and had broken through the perivascular spaces into the surrounding tissue, resulting in an injury of the brain with a secondary reaction on the part of the cerebral elements (fig. 2 *A*). The hemorrhages were not limited to the blood vessels and occasionally formed diffuse extravasations. Often only a few erythrocytes were found scattered between the nerve fibers. In the more severe involvement the red cells formed an irregular thin layer that completely covered the underlying tissue. These lesions seemed less injurious than the large destructive perivascular hemorrhages.

The perivascular spaces were greatly distended and presented rarefied tissue for a considerable distance around the vessel. The glial trabeculae that span the perivascular spaces appeared to have been torn away from the walls of vessels. Occasionally a few erythrocytes were seen scattered through these distended spaces.

Many of the capillaries and small arterioles under 50 microns in diameter showed still another change. This consisted of swelling and proliferation of their endothelial lining. The nuclei of the endothelial cells were so intensely swollen that they frequently overlapped one another and occluded the lumens of the vessels. Many of these nuclei became vacuolated, and their chromatin particles were displaced to the periphery. In some areas the process apparently continued with proliferation of the endothelial cells both inwardly to fill the lumens of the vessels and outwardly to produce extensive new vessel formation. This alteration of the vessels was widely scattered throughout the tissues but seemed more prominent within the vessels of the white substance. The rest of the element of the walls of vessels did not seem to participate in the process. The larger vessels remained more or less inactive and showed none of these endothelial changes. Evidence of inflammation was entirely lacking.

The hemorrhagic process, although present in both cases, was much more extensive in the second, in which the bleedings were often large and had caused fairly intensive damage of the brain. The vascular dilatation, perivascular distention and endothelial swelling and proliferation were present in about equal intensity in both cases. This endothelial alteration was described in 1929 by Winkelman and Eckel [2] under the name of proliferative endarteritis. These authors found these changes in certain cases of severe infection and toxemia. Their observations have since been substantiated by other investigators under similar circumstances.

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2. Winkelman, N. W., and Eckel, J. L.: Arch. Neurol. & Psychiat. **21**:863, 1929.

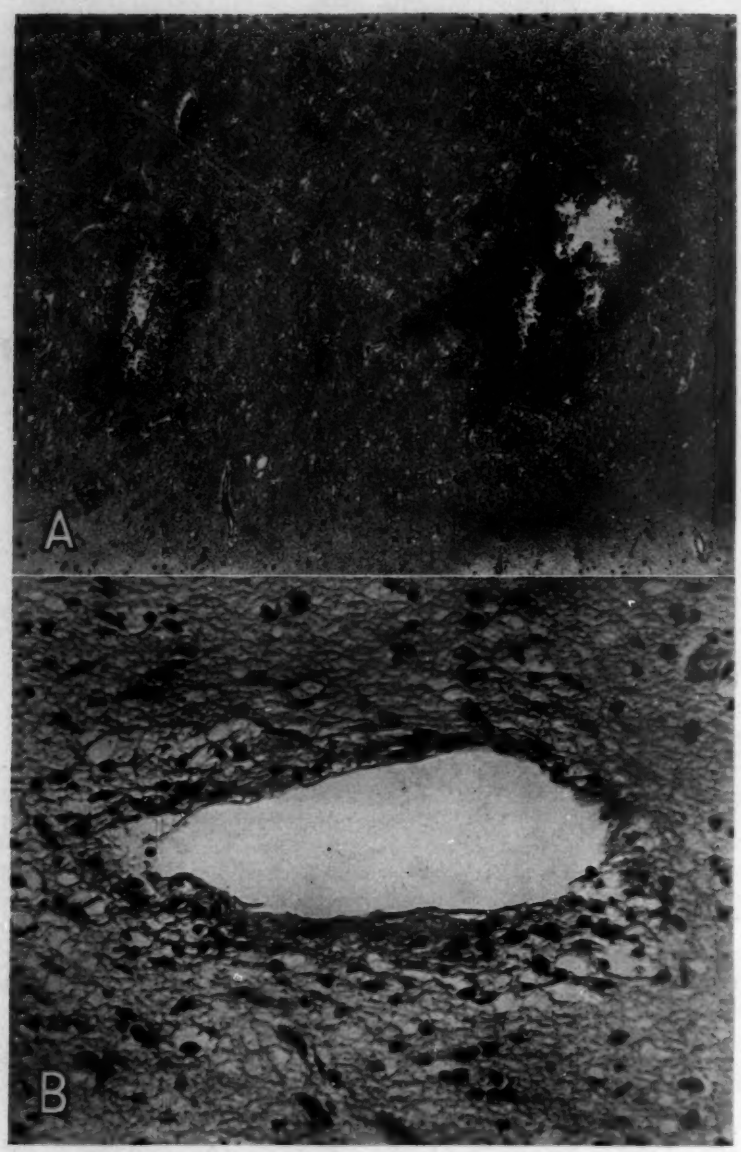


Fig. 2.—*A*, petechiae. Note their extensiveness and the associated injury to the tissue. *B*, photomicrograph of one of the smaller cysts. A fairly well developed glial wall completely surrounds the small cavitation.

Myelin Changes: The myelin sheaths presented a patchy type of demyelinization with the production of numerous small involved areas, scattered irregularly throughout the entire brain, excluding the cerebellum. Here and there, however, diffuse demyelinization was encountered, involving much larger regions of the white substance. All stages of destruction of myelin were observed, from fragmentation to granular disintegration. Many of the lesions were mild and revealed minimal alteration, while others showed complete destruction with formation of many moderate-sized unstained areas or cysts.

The presence of these cysts was striking. They varied in size and were usually empty or at most lined by a few fat granule cells of varied size and shape. These cells extended for some distance into the adjacent brain tissue. A few unaltered microcytes and many rod cells were present around these regions. The older cysts were lined by a fairly well developed wall of bipolar cells with elongated, darkly staining nuclei. This glial proliferation was most complete around the older cyst structures (fig. 2 *B*).

The extensive areas of encephalomalacia visible on gross inspection had resulted in the formation of many huge cavities, surrounded by softened tissue. The walls of these irregular cysts presented an interesting picture. In some regions they were composed of necrotic material invaded by a number of fat granule cells. In other areas the alterations in the tissue had affected to various degrees the macroglia, which showed active proliferation. A few of these large softenings were partially surrounded by a most marked glial reaction. The proliferating astrocytes presented irregularity in the shape and size of their nuclei, with giant cell formation and occasional mitoses. This cell growth diminished and tapered off into the surrounding brain tissue, which presented only moderate astrocytic proliferation.

The extensive areas of brain destruction were invariably surrounded by many of the smaller cysts and demyelinated foci already described. Most of them were limited to the white substance, although the larger lesions had extended into and through the cortex almost to the surface of the brain.

The destruction of myelin was most extensive in the second case, in which large cavities had been formed throughout the hemispheres. However, microscopic and macroscopic cavitation occurred in both brains. From the nature of these cavitations and of the cellular reactions associated with them, there can be no question that they occurred before death. That such lesions, even if of a lesser degree, would result in an irreversible process with permanent damage to cerebral function is obvious.

Cell Changes: Consistent specific changes in the ganglion cells have not been observed. In those regions where the huge cavitations had involved the cortex, ganglion cells were found showing various degrees of degeneration, as evidenced by fragmentation of their processes and vacuolation, disintegration and dissolution of some of the cell body. These cells showed partial to complete chromatolyses but no neuronophagy or satellitosis. Nerve cell changes did not occur throughout large cortical areas but were limited to only a few ganglion cells here and there within the dead brain tissue surrounding the areas of demyelinization.

The macroglia had undergone the most consistent alterations. There was a considerable increase in the neuroglia elements of both the cortex and white substance, being more extensive in the latter region. Few areas of the brain were exempt from this process, although the intensity varied greatly in different regions. In the areas of milder reaction the astrocytes were moderately increased in number, but their nuclei remained uniform, round and vesicular (fig. 3 *A*). The proliferation of the macroglia ranged from these mild responses to extensive reactions, in

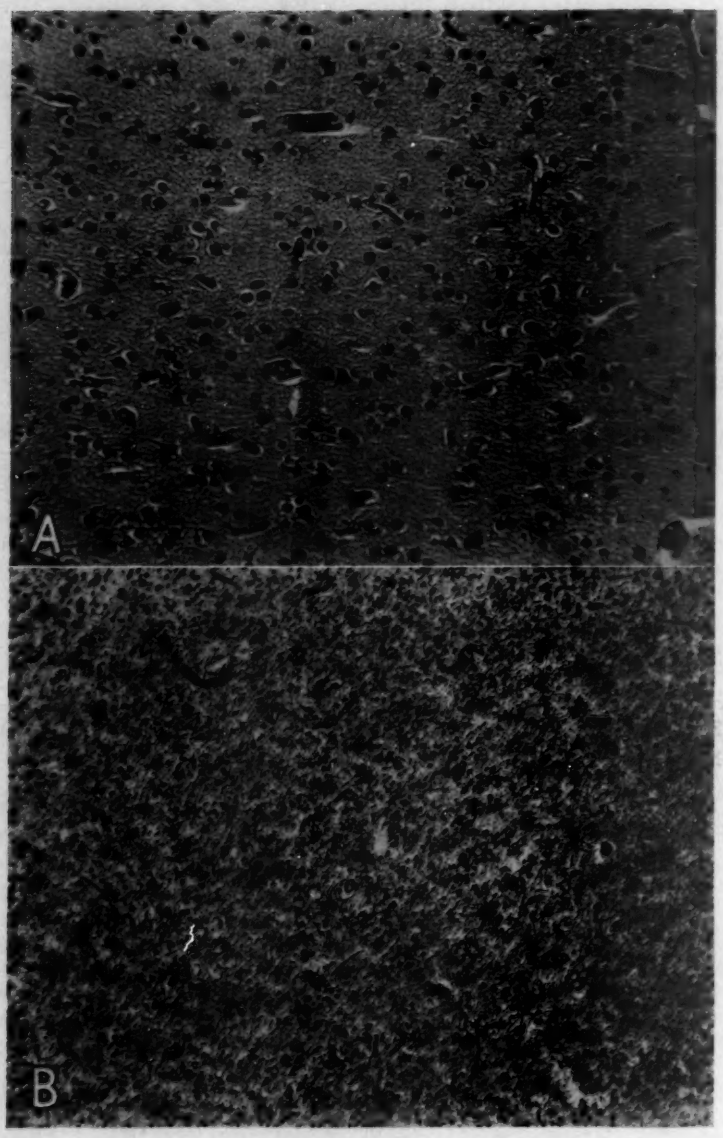


Fig. 3.—*A*, area of mild macroglial increase. Note the uniformity in size and shape of the cell nuclei. There is a beginning demyelinization of the surrounding tissue. *B*, extensive proliferation of the glial cells. This low power view illustrates the degree of tissue involvement and cellular proliferation.

which the cells were numerous and varied in structure from an elongated bipolar type with long intertwining processes to a huge type with giant nuclei filled with heavy chromatin granules (fig. 3 *B*). Many of the cells exhibited mitotic figures. Gliosis was prominent, giving these regions an increased fibrous appearance. This glial proliferation seemed independent of the destruction of myelin and occurred in many areas where the myelin sheaths were intact. When demyelinization had occurred, the astrocytic activity was accentuated, and with the ordinary stains one could make out all details of the astrocytic body with its numerous ramifying processes.

The macroglial reaction was often of a nodular type and resulted in the scattered appearance of many fairly well circumscribed clumps of glial tissue. The macroglial proliferation reached its greatest activity around the larger cysts, where the astrocytic proliferation seemed most active and the cells most dense. The smaller cavities were surrounded by less extensive but still definite glial collars composed of somewhat elongated cells. The intensity of the reactive process gradually decreased as the fields were selected farther and farther from the cavities.

The microglia revealed varying degrees of activity, showing the most alterations in the vicinity of the cavities and within the severely demyelinated areas. In these cells the most frequent change was a transformation into so-called rod cells. These cells were frequently observed intermixed with the macroglia cells in the areas of increased cellularity.

The oligodendroglia showed mild swelling of cells but little or no increase in cell number.

The cellular changes occurring within these tissues were most significant, especially if one considers the reaction on the part of the macroglia. These cells showed striking proliferation in both cases but more marked in the first. The alterations were generalized and involved all areas of the brain but were most accentuated in the temporoparietal regions and around the various cyst formations. The cortex as well as the white substance was involved.

## COMMENT

It is well recognized that hypoglycemia will produce widespread, bizarre and often persistent clinical findings, all of which point strongly to some definite change or changes within the central nervous system. As a rule the symptoms, even though extensive, are transitory if treated early. However, when the attacks are severe and prolonged, the patient may go into a state characterized by continuing neurologic disabilities of one sort or another, or his condition may progress to a fatal termination even in the face of attempts to control the level of the blood sugar. Numerous examples of such occurrences are now on record. Wolf, Hare and Riggs [3] observed 2 cases of hypoglycemia, in one of which the condition ended fatally, while in the second there were residual signs of hemiplegia for days. Bast, Schmidt and Sevringhaus [4] studied

3. Wolf, A.; Hare, C. C., and Riggs, H.: Bull. Neurol. Inst. New York **3**: 232, 1933.

4. Bast, T. H.; Schmidt, E. R., and Sevringhaus, E. L.: Acta chir. Scandinav. **71**:82, 1932.

a 44 year old man in whom neurologic findings persisted for five days after adequate feeding had been instituted. Darrow [5] reported 2 cases of permanent mental deterioration associated with convulsions and hypoglycemia. Blau, Reider and Bender [6] cited the case of a 49 year old man suffering from repeated severe attacks of hypoglycemia, in whom there developed progressive and permanent extrapyramidal and cortical signs. In this clinic there are records of permanent neurologic and mental disturbances resulting from recurrent attacks of hypoglycemia. Such reports and the occasional record of death make it evident that hypoglycemia must produce widespread severe damage to the central nervous system. From the present studies these lesions may be divided into three large groups:

1. Cerebral petechiae (fig. 2 *A*). These vary from small discrete extravasations scattered irregularly throughout the brain substance to large hemorrhages that tear into the surrounding tissues and produce definite cerebral damage.

In our earlier studies Lufkin and I were particularly impressed by these hemorrhages, to the exclusion of the other possible changes, and described old and new petechiae in all the cases studied. These hemorrhagic lesions have been found and described by others in both human and animal tissue (Schereschewsky and others; [7] Morsier and Mozer; [8] Lindsay and others [9].

2. Large areas of encephalomalacia, demyelinization and cyst formation (figs. 1 and 2 *B*). There can be no doubt even from the gross inspection of the brains in the present cases that the huge areas of softening must have resulted in functional damage and have been responsible in part for the progressing syndrome. It is curious that the vessels surrounding these softened areas were entirely intact and gave no evidence of thrombosis or of arteriosclerotic changes such as one might expect in a diabetic patient. Since both patients lived for over a week in a comatose state, sufficient time had elapsed for these extensive alterations to become visible. Lindsay and his co-workers [9] described some cystic spaces in the brain of their patient who died during a hypoglycemic reaction. These authors, however, believed that the cysts were due to pressure from fluid accumulating within the cerebral fissures.

The patchy and diffuse demyelinization as well as the formation of numerous small cysts are by no means without damaging results to the

5. Darrow, D. C.: Am. J. Dis. Child. **51**:575, 1936.

6. Blau, A.; Reider, N., and Bender, M. B.: Ann. Int. Med. **10**:910, 1936.

7. Schereschewsky, N. A.; Mogilnitzky, B. N., and Gorjaewa, A. W.: Endokrinologie **5**:204, 1929.

8. Morsier, G., and Mozer, J. J.: Ann. de méd. **39**:474, 1936.

9. Lindsay, J. W.; Rice, E. C.; Selinger, M. A., and Mish, K. H.: Ann. Int. Med. **10**:1892, 1937.

nervous tissue. These small glia-lined cavities were described in our earlier paper,[1] and at that time their origin was doubtful. It was believed that they might represent the remains of an old hemorrhage. In view of the present studies, that interpretation probably was fallacious. The cysts, no doubt, are the result of a complete demyelinization of patchy areas of brain tissue associated with a secondary reaction of the neuroglia and the formation of a glial wall. Intermediate stages in their formation were observed in the present studies.

3. Diffuse, generalized glial proliferation and extensive gliosis around the areas of softening (fig. 3). This alteration of brain tissue is perhaps one of the most striking. The astrocytes have proliferated in all areas of the brain with the formation of diffusely cellular regions containing macroglia cells of all shapes and sizes, including giant cells, bipolar cells, pyknotic cells and mitotic cells. In many regions the reaction is only mild, but in others it is truly massive. No reason could be found for this regional severity of the reactive process. Around the softened brain tissue and around the small cysts, the macroglia cells have formed definite glial walls. Certain portions of the large areas of encephalomalacia were surrounded by most extensive astrocytic proliferation. Intermixed with these cells were numerous microglia cells in various stages of transformation to fat granule cells. Morsier and Mozer [8] described a striking glial proliferation which was chiefly perivascular, although it did occur diffusely through the hemispheres. Their patient died from insulin hypoglycemia used in the treatment of morphinism. Hemorrhages were also observed through their sections.

It is apparent that any single one or any combination of these cerebral alterations is sufficient to produce severe, lasting injury of the brain, permanent functional damage or death. Such findings, in scattered cases of hyperinsulinism, have now been mentioned periodically in the literature.

While the literature contains few reports of disasters from insulin shock therapy, it seems to be no far fetched conclusion that lesions like those described in this article might well occur in such circumstances. I know personally of 2 cases in which such therapy terminated disastrously, cases which have not been reported in the literature. This seems a rather large risk to run while the mechanism of the therapy is so poorly understood and the percentage of cures (in schizophrenia, for example) remains relatively low and the statistical significance rather uncertain.

Until further unfavorable clinical outcomes and pathologic descriptions of brains from persons dying after insulin shock therapy are available, one would hardly advise the widespread discontinuance of

insulin shock therapy, although in this clinic there has resulted from the observations reported herewith an attitude of reserve and conservatism.

SUMMARY

In the study of the cerebral lesions in 2 cases of hypoglycemia, extensive changes were observed within the vessels, the myelin and particularly the glia. The possibility of such injury to tissue should make one cautious in the use of hypoglycemia as a therapeutic agent.

# EXPERIMENTAL PRODUCTION OF TUMORS IN THE BRAINS OF WHITE RATS

ARTHUR WEIL, M.D.
CHICAGO

With the advent of synthetic carcinogenic substances and the resultant production of manifold neoplasms of the different organs of laboratory animals, it would have been quite natural if the neuropathologist, too, should have availed himself of this new progress in tumor research. If the literature until recently contained only scattered reports of negative results, it does not reflect the actual state of affairs, because many efforts to produce glioma experimentally had failed and were not published. Two of such disappointing experiments were reported by Roussy, Oberling and Raileanu [1] and by Oberling, Guérin and Guérin.[2] In the first publication small pieces of gauze saturated with coal tar were applied to the surface of the rabbit brain, and severe necrosis but no formation of a tumor occurred. In the second series of experiments crystals of dibenzanthracene were implanted in the cortex of the rat's brain, but no reaction of the brain tissue around this implant was observed. Injections of oily solutions of this carcinogenic substance likewise gave negative results. It was noted, however, that adenoma of the hypophysis was produced in these experiments.

Despite these disheartening results, the attempt was made to study the blastogenic properties of lard solutions and cholesterol combinations of dibenzanthracene,[3] of methylcholanthrene and of the more recently discovered styryl 430 (2 [p-aminostyryl] 6 [p-acetylaminobenzoylamino] quinoline methoacetate).[4] This substance, which had been originally synthesized for combating trypanosomal infections, produced large tumors when injected subcutaneously into rats. These tumors developed from the original phagocytic tissue and appeared as pleo-

From the Institute of Neurology, Northwestern University Medical School.

1. Roussy, G.; Oberling, C., and Raileanu, C.: Compt. rend. Soc. de biol. **104**:763, 1930.

2. Oberling, C.; Guérin, M., and Guérin, P.: Compt. rend. Soc. de biol. **123**: 1152, 1936.

3. Burrows, H.; Hieger, I., and Kennaway, E. L.: Am. J. Cancer **16**:57, 1932. Shear, M. J.: ibid. **26**:322, 1936.

4. (*a*) Browning, C. H.; Cohen, J. B.; Cooper, K. E.; Ellingworth, S., and Gulbransen, R.: Proc. Roy. Soc., London, s.B **113**:300, 1933. (*b*) Browning, C. H.; Gulbransen, R., and Niven, J. S. F.: J. Path. & Bact. **42**:155, 1936.

morphic sarcoma. Dr. C. H. Browning enabled me to obtain a sufficient supply of styryl 430, and the Eastman Kodak Company supplied 1, 2, 5, 6-dibenzanthracene and methylcholanthrene.

The accompanying table reviews the different experiments which were carried out and the results.

*Intracerebral Injections into White Rats*

| Material Injected | Rats | Survival, Days | Results |
|---|---|---|---|
| Dibenzanthracene-lard..... | 6 | 187-217-217-228-229-229 | Granuloma in 6 cases; carcinoma in 1 case |
| Dibenzanthracene-cholesterol.. | 2 | 185-201 | No growth |
| Methylcholanthrene-lard..... | 3 | 161-207-219 | Granuloma in 2 cases; no growth in 1 case |
| Styryl 430..... | 5 | 7-7-125-152-180 | Formation of tumors in last three rats |
| Lard (control)..... | 2 | 216-239 | Granuloma |
| Cholesterol (control)..... | 2 | 182-210 | No growth |

## TECHNIC

Male and female white rats of the laboratory stock were used, weighing from 120 to 180 Gm. Under combined morphine, pentobarbital sodium and ether narcosis a longitudinal section was made through the skin covering the skull, at the right, about 5 mm. distant from the midline and about 15 mm. in length. The underlying periosteum was removed, and a small hole about 1 mm. in diameter was driven through the skull in the parietal region, with the help of a dental drill. The substance to be tested was injected through a fine needle attached to a tuberculin syringe, and from 0.10 to 0.15 cc. was injected into the brain, with the needle pointing in different directions in the different experiments. The skin was then closed with silk sutures. Aseptic precautions were taken throughout the operation.

The lard solutions of dibenzanthracene and methylcholanthrene were prepared as follows: Five grams of lard was dissolved in about 20 cc. of acetone on the electric heater, and 50 or 100 mg. of the substance to be tested was added (making a 1 or 2 per cent solution in lard). After it had been dissolved, the acetone was evaporated, and the resulting oily substance was sterilized at 110 C. Before injection it was warmed to about 40 C. The styryl 430 was injected in a 1 per cent suspension in saline solution, which before being used for injection was sterilized by short boiling or the styryl was dissolved in distilled water. Cholesterol compounds were prepared as acetone solutions as described, and after evaporation of the acetone the resulting mixture was heated to the melting point. Small crystals were implanted through the drill hole into the brain.

While the rats recovered quickly after the injections of dibenzanthracene and methylcholanthrene, they were acutely ill in the experiments with styryl 430. They never regained the weight of the control rats, and after about seventy days the weight curve flattened out. At the end of each period given in the table a rat was killed by decapitation, and the brain was immediately fixed in solution of formaldehyde U. S. P., embedded in paraffin and cut serially. The staining methods employed were the cresyl violet, the Davenport (for glioma), the Holzer and the Perdrau. From some brains frozen sections were prepared and stained by Cajal's and Hortega's methods for microglia.

RESULTS

*Implantations of Dibenzanthracene-Cholesterol Crystals and of Choiesterol Crystals.*—These implantations did not lead to any marked reaction of the glia in the environment. As in the experiments of Oberling, Guérin and Guérin, an empty cavity was found in place of the crystals (which had been dissolved during the process of embedding the brain in paraffin). It was surrounded by compressed brain tissue without marked gliosis. At the bottom of this cavity the remains of a small area of organized hemorrhagic softening was found.

*Injections of Dibenzanthracene-Lard, of Methylcholanthrene-Lard and of Lard.*—In these experiments one has to differentiate between the effect of the solvent, lard, alone and the effect of the carcinogenic substance. In all the experiments of this group there was uniformly the production of granuloma as a reaction to the invading foreign body lard. On the removal of the brain one could see grossly that the oily substance had invaded the ventricles and following gravity had accumulated in the subarachnoidal spaces at the base of the brain. The upper end of the wound made by the injection had healed without any marked reaction, leaving a small glia scar only. However, where the substances which had been injected had been arrested, at the posterior poles of the lateral ventricles, at the aqueduct, in the fourth ventricle and its lateral recesses and in the pia-arachnoid covering the cerebellum and pons, a remarkable reaction had occurred. The ependymal lining together with the subependymal glia had proliferated and formed small tumors which protruded into the lumens of the ventricles. The tumors contained slitlike empty spaces, lined by elongated multinuclear cells. Between them there was an abundant proliferation of glia cells which had formed many elongated forms and giant astrocytes. In other places rosettes were found, lined by large cells, rich in cytoplasm, which extended their processes into the lumen. The reaction of the pia-arachnoid likewise produced slitlike cavities lined by multinuclear cells. Besides, there was active proliferation of fibroblasts with a considerable increase in collagenous connective tissue (figs. 1 *A* and 2). Injections of lard alone produced the same marked reaction as combinations of lard with dibenzanthracene and methylcholanthrene, and the effect did not seem to be more intensified in the latter experiments than in the lard controls.

Among the pathologic conditions of the human brain, granuloma like that described is rare. In the collection of this laboratory is a cyst of the third ventricle which shows a histologic structure corresponding exactly to that of the lard granuloma. In its wall slitlike empty cavities were found, surrounded by large, elongated multinuclear cells, rich in cytoplasm, and there was a considerable increase in loosely arranged coarse collagenous connective tissue fibers.

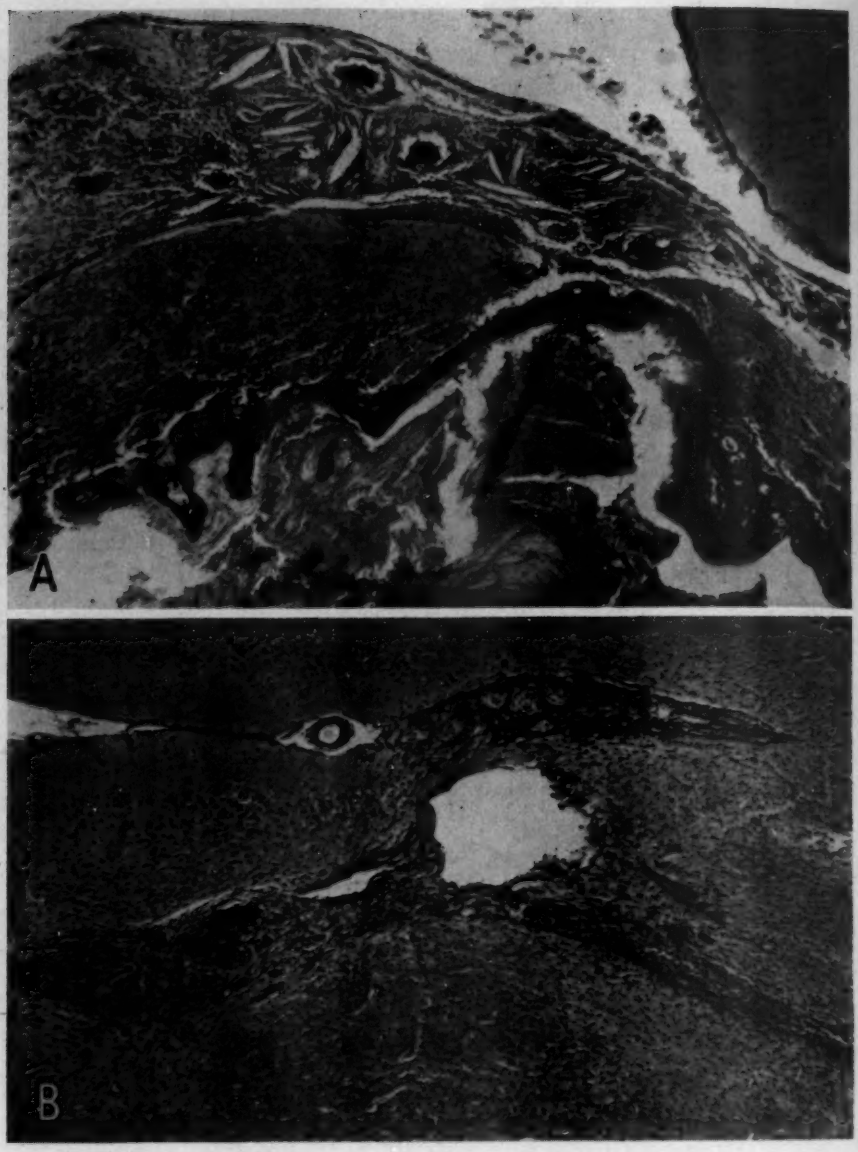


Fig. 1.—*A* is a horizontal section through the midbrain, which shows the results of an injection of a lard solution of dibenzanthracene observed seven and a half months after the injection. The papillomatous processes of a squamous epithelial carcinoma are seen invading a cystic cavity filled with a débris of tumor cells. Surrounding the tumor is a marked glial proliferation. In the pia-arachnoid a granuloma has formed in response to the lard. Van Gieson stain. Magnification, × 103. *B* is a horizontal section through the brain, which shows the result of an injection of styryl 430, observed five months after the injection. In the caudal end of the longitudinal fissure a meningioma-like proliferation is seen. Below, a gliomatous proliferation extends through the whole length of the photomicrograph. Cresyl violet stain. Magnification, × 43.

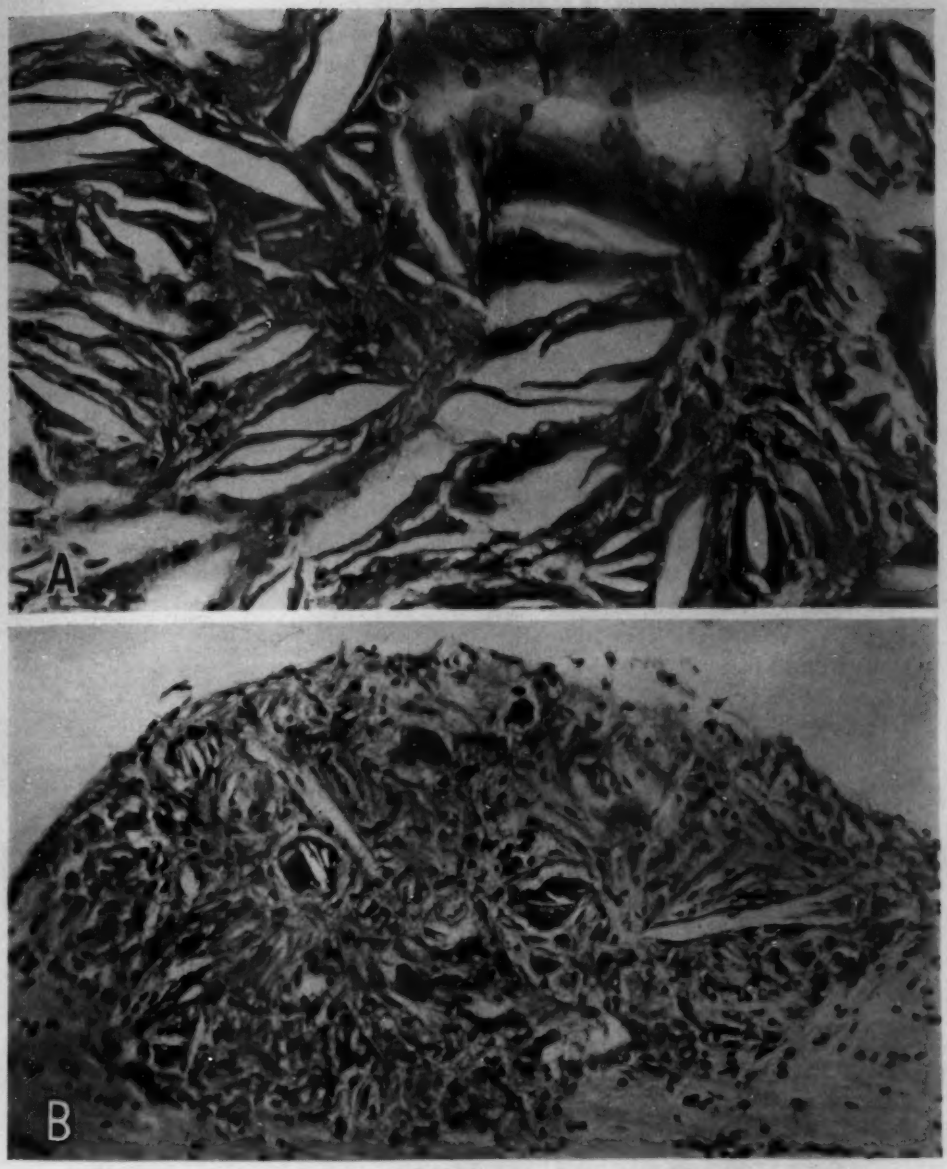


Fig. 2.—*A* demonstrates the histologic structure of a lard-induced granuloma of the pia-arachnoid developing as a result of an injection of a lard solution of dibenzanthracene observed seven months after the injection. Acicular slits are seen, surrounded by multinuclear giant cells. Van Gieson stain. Magnification, × 333. In *B* is seen a granuloma in the lateral ventricle caused by an injection of lard, observed seven months after the injection. Cresyl violet stain. Magnification, × 190.

In one rat only was tumor formation found which could be attributed to the specific effect of dibenzanthracene. This was a male rat which had been killed seven and a half months after the injection of 0.1 cc. of the dibenzanthracene-lard mixture. Its weight had increased from 147 to 398 Gm. At autopsy the internal organs appeared to be grossly normal. After the brain was cut horizontally, a large cavity was found, which extended through the midbrain to the base of the skull and was filled with a greenish soft mass. The surface of the bone at the point of contact with the cyst was rough and eroded. In histologic sections it was found that this cavity seemingly included the extremely widened posterior horn of the right lateral ventricle, that it compressed the whole right hemisphere forward approximately to the anterior commissure and that it had destroyed part of the right midbrain. The wall of the cavity was lined by a papillomatous growth which extended its processes into the cavity and which invaded the surrounding brain tissue. The ependymal lining of the ventricle and the mesothelial lining of the invaded pia-arachnoid showed proliferation. At points of transition the nuclei of the ependymal cells became clear and increased in number, and in the underlying subependymal tissue there was mild proliferation of glia cells. The papillae of the tumor extending into the cavity were surrounded by coarse fibrils, concentrically arranged. The cellular arrangement was of epidermoid type, and in the outer zones the histologic appearance resembled somewhat squamous epithelium (fig. 1 *A* and 3).

In the collection of this laboratory a suprasellar squamous epithelial papillary cyst and a tumor were found which resembled histologically the dibenzanthracene carcinoma. The tumor was a large cyst situated within one cerebral hemisphere, where it was in contact with the lateral ventricle. Its wall was lined by papillary protrusions, which consisted of epithelioid cell masses surrounding cavities filled with blood vessels and a loose connective tissue stroma. Since no primary carcinoma had been found elsewhere in the body, the growth could not be considered as a metastasis, and the diagnosis of a rare primary epithelial tumor of the brain was made.

*Injections of Styryl 430.*—Experiments with this substance produced more uniform and specific results than the preceding ones. In the first rat, surviving four months, it was evident that a substance had been found which produced tumors in different structures of the rat brain. Wherever the fine particles of the dye had come into contact with brain tissue, a proliferating reaction occurred which was entirely different from the reaction of brain tissue to inactive foreign bodies or to damage by wounds or hemorrhage. In describing these reactions, one has to differentiate between the scar proper which was produced at the point of the injection and the reaction of the tissue remote from this point. The particles of dye were found along the walls of the ventricles,

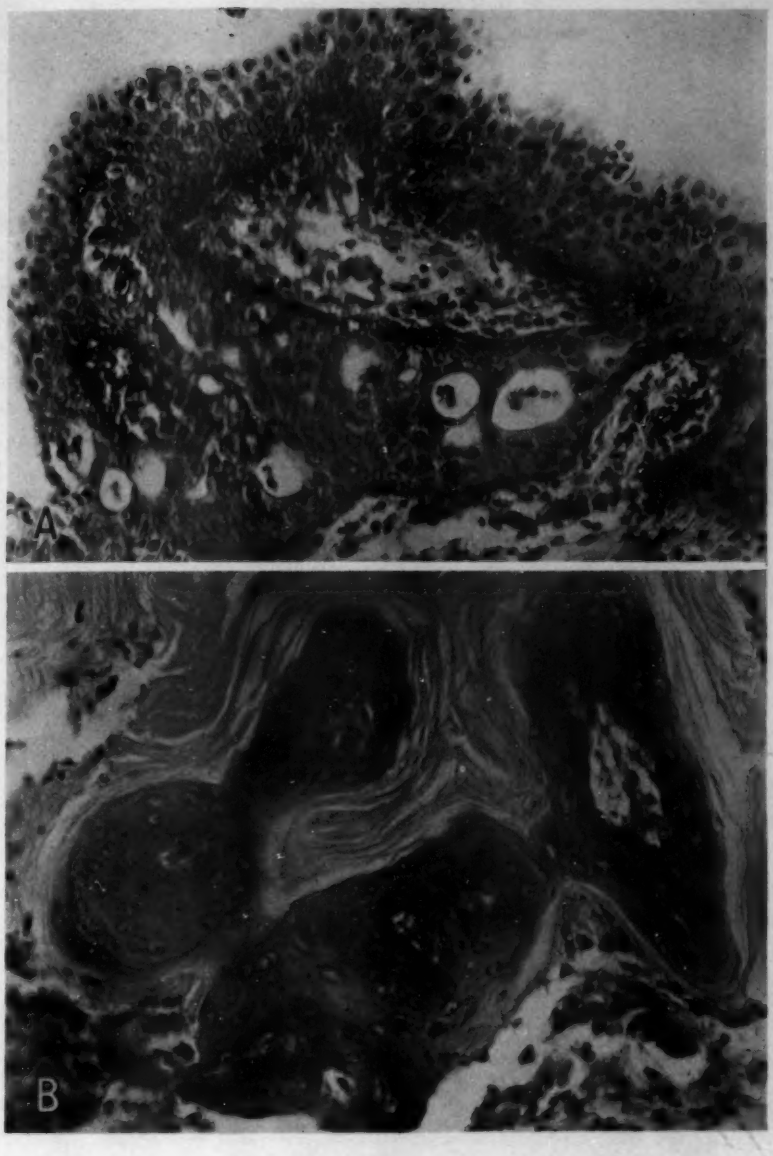


Fig. 3.—*A* shows a higher magnification of the carcinoma in figure 1 *A*, demonstrating the structure of a papilla with a central core of loose tissue surrounded by squamous epithelium. Magnification, × 224.5. *B* represents the same experiment as figure 1 *A*. Islands of tumor cells are seen, surrounded by concentrically arranged fibrillary substance. Van Gieson stain. Magnification, × 224.5.

in the pia-arachnoid covering the surface of the brain and lining the sulci, the brain tissue surrounding the dye deposits, the roots of the cranial and spinal nerves and finally the hypophysis.

In order to study the histologic reaction better, it was necessary to decolorize the dye by treating the sections freed from paraffin with a 0.5 per cent solution of potassium permanganate for about five minutes. They then were left in a mixture of equal parts of oxalic acid and sodium bisulfite (5 per cent solution of each) for a time, carefully washed and stained as usual.

The retardation of growth beginning after about two months, which has been mentioned, suggests that at this period the brain and the hypophysis must have been already markedly affected. Two rats which died after seven days already showed the stimulating effect of the dye on tissue growth in the form of a proliferation of glia, connective tissue and Schwann's cells but only to a very mild degree in the environment of the dye deposits. The latter were contained in large phagocytic cells, rich in cytoplasm. But penetration of the finer dye particles into glia cells of the environment was not yet very definite at this early stage.

In the rat killed at four months, however, the neoplastic process was definitely established. In the brain tissue proper, which had been invaded by the dye, changes were noticeable which reminded one very much of the early stages of glioblastoma multiforme in men. The zone directly adjacent to the dye, containing phagocytic cells, had a loose structure (figs. 1 *B* and 4 *B*). In Davenport-stained preparations large, elongated cells could be seen, which were argyrophil and resembled closely the "unipolar" and "bipolar" "spongioblasts" (fig. 5 *B*). In the area surrounding this neoplastic transformation there was marked proliferation of astrocytes, which formed peculiar giant branching forms (fig. 5 *A*). The cells of the oligodendroglia in this outer zone were increased in number and showed a tendency to form oval nuclei, which was similar to their reaction in the environment of glioblastoma multiforme. The center of the tumor itself contained a dense accumulation of argyrophil cells of manifold sizes and shapes: elongated, "spongioblast"-like forms, similar to those in the loose outer zone; large round or polygonal cells rich in cytoplasm were intermingled with them (fig. 4 *A*). No proliferation of blood vessels was noticeable in these regions.

Wherever the dye had come into contact with the wall of the ventricles there was marked proliferation of ependymal cells, which were intermingled with elongated forms of glia cells and astrocytes. Numerous small tumors were formed in this way, which protruded into the lumens of the ventricles.

Pia-arachnoid invaded by styryl 430 responded with massive proliferation of fibroblasts and an increase of connective tissue fibers.

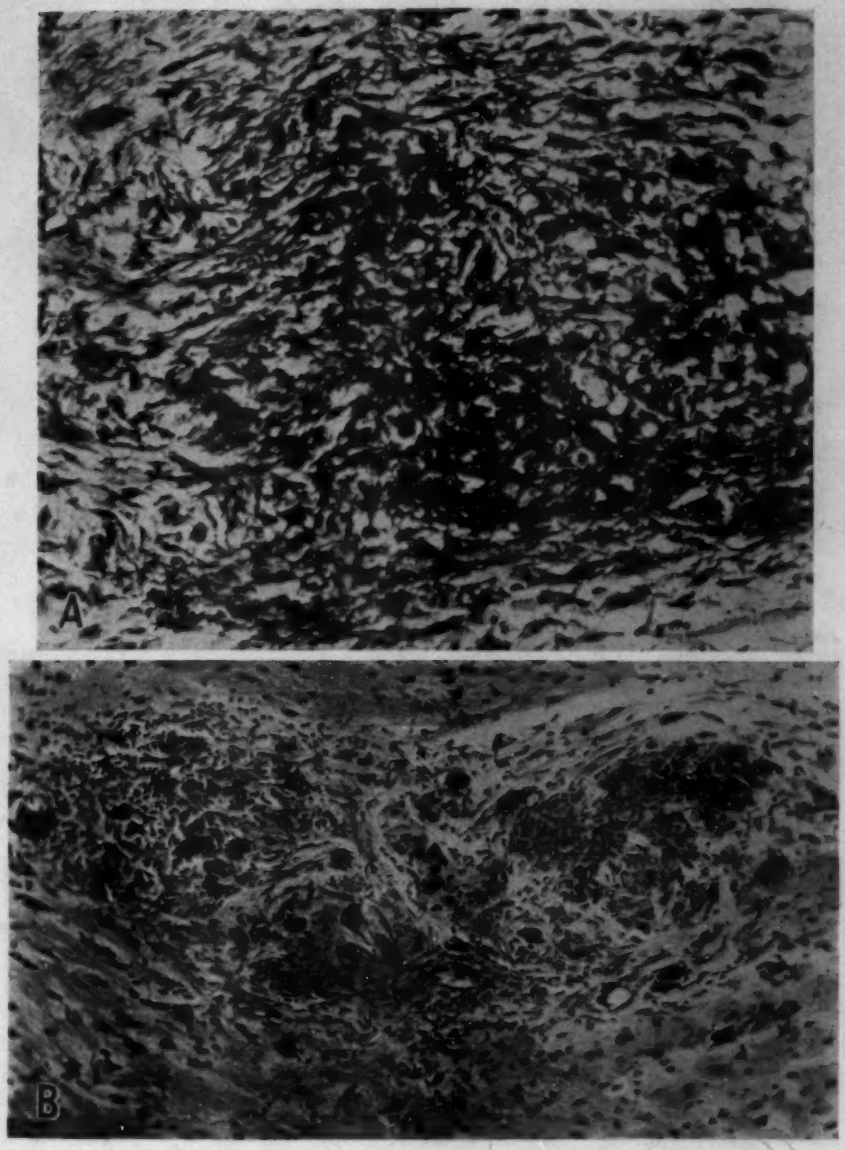


Fig. 4.—*A* is a glioma formation with densely accumulated polar cells caused by an injection of styryl 430, observed five months after the injection. Davenport stain. Magnification, × 308.5. *B* represents the same case as *A*. It shows the glioma formation with a center of dye-containing phagocytic cells, surrounded by a loose proliferation of polar cells. Cresyl violet stain. Magnification × 137.

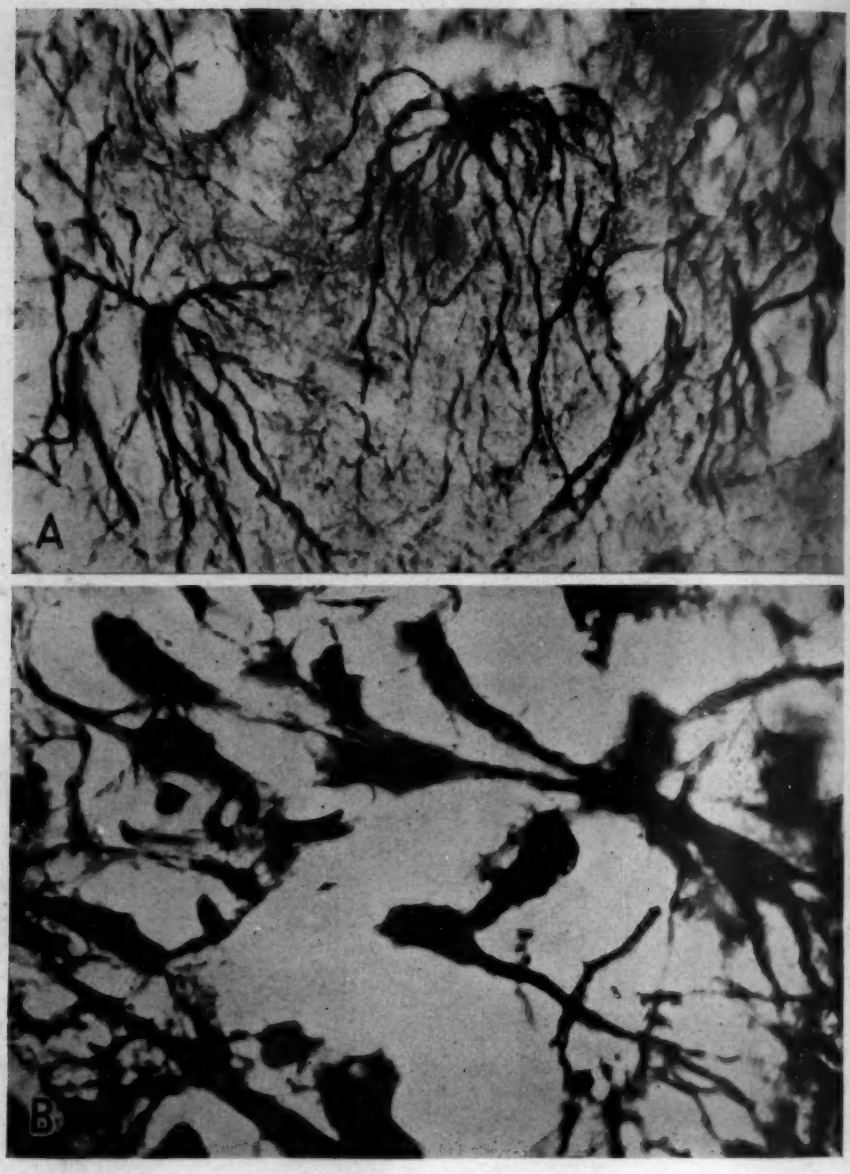


Fig. 5.—*A* shows giant branching astrocytes in the outer zone of a gliomatous proliferation caused by an injection of styryl 430, observed four months after the injection. Cajal's stain. Magnification, × 650. *B* shows tumor cells resembling "unipolar spongioblasts." Davenport stain for glioma. Magnification, × 1100.

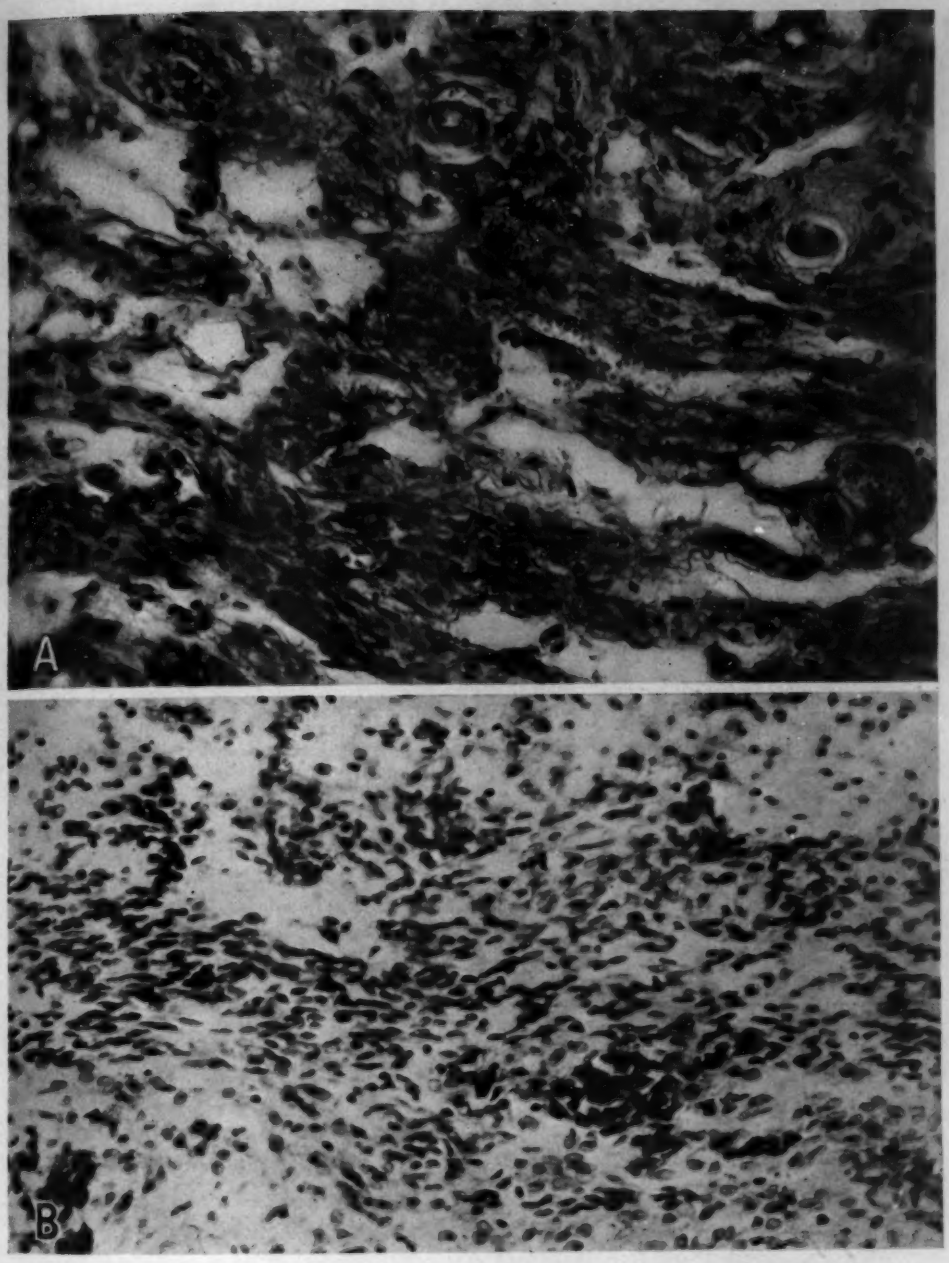


Fig. 6.—*A* shows the result of an injection of styryl 430, observed five months after the injection—a meningioma-like formation with elongated cells forming whorls around blood vessels and clusters of larger cells intermingled with coarse collagenous connective tissue fibers. Van Gieson stain. Magnification, × 300. *B* represents the outer zone of the medulla oblongata, cut horizontally, showing proliferation of long, oval cells of the Schwann sheath cell type, intermingled with proliferated glia. Cresyl violet stain. Magnification, × 300.

Besides, there were accumulations of larger, epithelial-like cells, rich in cytoplasm and indefinite in outline, which also were arranged in larger clusters. Around smaller blood vessels were seen whorled formations of elongated cells intermingled with coarse connective tissue fibers. The histologic picture as a whole reminded one very much of a meningioma formed in a human brain (fig. 6*A*).

The dye had apparently passed through the aqueduct into the fourth ventricle and from here through its lateral recesses into the pia-arachnoid covering the base of the brain and the spinal cord. In the latter it was mostly condensed at the posterior surface and faded out gradually toward the thoracic segments. Where it came into contact with cranial or spinal nerve roots it stimulated overgrowth of Schwann's sheath cells and fibroblasts, which, following the course of the nerve fibers, had a tendency to extend in long lines of oval or spindle-shaped nuclei (fig. 6*B*). Again one could compare this reaction with a neurinoma of a cranial nerve in a human brain. In the spinal cord the outer, Held's, zone adjacent to the dye responded with an increase in glia nuclei and with the formation of large fibrillary astrocytes.

One may thus shortly summarize the results of the experiments with styryl 430 in stating that each tissue which came in contact with the dye formed its own, specific neoplasm: the subcutaneous tissue, sarcoma; the glia, glioblastoma; the pia-arachnoid, meningioma, and the ependymal lining of the ventricles, the tumors peculiar to these structures.

## COMMENT

Browning, Gulbransen and Niven,[4b] in describing their experiments with styryl 430, stated that the first stage of the formation of the subcutaneous tumor is the formation of phagocytic tissue, which after a stationary period suddenly shows acceleration of growth, hardening of the soft tissue and transformation into a sarcoma. It seems that six months or more have to elapse before this transformation takes place. In my experiments the brain tissue had seemingly overcome this inactive period of organization of phagocytic tissue within four months. Further detailed investigations have to be made in order to decide more exactly when neoplastic transformation begins. From the change in the growth curve, its flattening out around the beginning of the third month, one might predict that these physiologic changes indicate changes in the histologic character of the brain and of the hypophysis.

The hypophysis had been neglected in the earlier experiments. In the later series it was embedded in paraffin, and sections were stained. Preliminary studies of the rats into whose brains lard and dibenzanthracene had been injected did not confirm the observation of formation of adenoma reported by others.[2] In the rats into whose brains styryl

430 had been injected, however, neoplastic transformation took place in the pars nervosa and the glandular portion, which will be reported in detail later.

As to the tumor-like proliferation produced by lard, it should rather be classified as a granulomatous reaction to the presence of the foreign body. It should be of interest to test the reaction of brain tissue to the injection of other lipoids. There can be no doubt, however, that styryl 430 produces true neoplastic transformation of the different structures of the central nervous system. It is of considerable interest to study the reaction of the different types of cells to the carcinogenic substance dibenzanthracene and to the sarcogenic styryl 430. In the first case a true carcinoma was produced, which became invasive and destroyed the surrounding brain tissue. In the second case gliomatous tumors and meningioma-like formations arose, which did not form metastases but which were produced wherever the dye came into contact with the different tissues. It was interesting to observe that in the rat receiving the dibenzanthracene-lard mixture, besides the tumor produced by the specific substance, granuloma occurred as a reaction to the lard (fig. 1 *A*). It is without any doubt that the tumors which were described were not accidental findings. In experiments with white rats extending over more than six years a spontaneous tumor of the brain has never been found in this laboratory, and no account of one could be found in the literature. One might discuss the question, however, whether the carcinoma in the rat receiving the benzanthracene-lard mixture did not arise from the periosteum, the bone of the skull or the skin and whether accidentally small particles of these structures had not been carried through the operating wound into the brain. On the other hand, a close study of the ependymal lining of the part of the ventricular system and of the mesothelial lining of the arachnoidal membrane in which the tumor had been found showed gradual neoplastic transformation with formation of papillary protrusions. Why only 1 of 6 rats should have responded with carcinoma while all of them produced lard granulomas cannot be explained at present.

The comparability of these experimental tumor formations with tumor formations in the human brain is so striking that one is tempted to assume that the latter are due to a similar cause, i. e., to stimulation of neoplastic transformation by chemical substances. At present theories concerning the formation of glioma are still under the influence of Cohnheim's theories of the misplacement of embryonic remains. It was conceded previously [5] that certain tumors, such as medulloblastoma, arising from the roof of the fourth ventricle or the rest of the cranio-

5. Weil, A.: Textbook of Neuropathology, Philadelphia, Lea & Febiger, 1933, p. 239.

pharyngeal duct may support such a working hypothesis. But this explanation certainly does not fit the great variety of glioblastomas, and attempts to construct germinal centers for them within the adult brain are doomed by the mere fact of their ubiquitous origin. The experiments reported may also contribute to the long-standing argument as to whether neurinoma of the cranial nerves is schwannoma or fibroblastoma. The example of styryl 430 shows that the tissue which is affected acts as a whole and that its different constituents participate in the neoplastic transformation.

One may apply to these experiments the conclusions of Dunning, Curtis and Bullock [6]: "The potency for malignancy must be a universal cell characteristic and the histogenesis of these tumors is determined by the fortuitous exposure to the irritant of the various types of cells."

SUMMARY

Injections of lard solutions of 1, 2, 5, 6-dibenzanthracene and of methylcholanthrene led to the formation of typical granulomas, which were also produced by injections of lard alone.

One case was noted in which injection of the dibenzanthracene-lard mixture produced a malignant carcinoma besides granuloma.

Crystals of cholesterol or of dibenzanthracene-cholesterol did not produce any neoplastic reaction after implantation into the rat brain.

Injections of solutions of styryl 430 produced, after about four months, neoplastic transformation of the different tissues which had come into contact with the dye. The different types of tumors are described as resembling glioma, meningioma and glioma of the ependymal lining of the ventricles.

6. Dunning, W. F.; Curtis, M. R., and Bullock, F. D.: Am. J. Cancer **28**:681, 1936.

# MEDULLOBLASTS OF THE INFANT BRAIN

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Fischl [1] in 1899 linked the small round perivascular cells which he found in the subventricular recesses of infant brains to encephalitis congenita. He believed the cells to be inflammatory ones, which supported the contention of Virchow that the fat in infant brains was evidence of encephalitis. Ranke [2] later observed similar cells in small round foci and in the perivascular spaces of the subventricular areas in fetal brains. He recognized them as germinal foci but thought that they disappeared at birth in normal brains. Wohlwill [3] found them regularly in the postpartum month and regarded only those still present in later months as pathologic, perhaps related to syphilis. Roback and Scherer [4] in their examination of many children's brains discovered that the germinal foci disappear at various times. Those along the posterior lateral ventricle are not found after four months, while those in the anterior horn of the lateral ventricles persist to eight months and those in the central areas to five months.

In the present study the small dark round nuclei of the outer, marginal layer of the cerebellum, of the cerebral marginal layer and of the subventricular foci have been regarded as medulloblasts. Not only do the absence of stainable cytoplasm and the size of the nuclei relate the cells morphologically to the medulloblasts of medulloblastomas but the cells are clearly the progenitors of the neuroblasts and spongioblasts which may be found near them. Moreover, the medulloblasts are more tenacious and more widespread than has hitherto been supposed. The brains in which they were observed were those of infants varying in age from birth to 2 years. The sections were taken from the basal ganglions, the cerebellum and from different parts of the cortex.

## OBSERVATIONS

*Cortex.*—A few medulloblasts may form the cerebral marginal layer during the first postpartum month. The layer is more apt to be found in the temporal lobe than elsewhere, particularly along the uncus.

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From the Richmond Memorial Hospital.

1. Fischl, R.: Jahrb. f. Kinderh. **49**:58, 1899.
2. Ranke, O.: Beitr. z. path. Anat. u. z. allg. Path. **47**:63, 1909.
3. Wohlwill, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **68**:384, 1921.
4. Roback, H. N., and Scherer, H. J.: Virchows Arch. f. path. Anat. **294**: 365, 1934.

Medulloblasts are also scattered in the first layer, but small groups or foci of them are found only in the subiculum, from which they disappear before seven weeks. In the groups of ganglion cells of the second layer of the subiculum the medulloblasts are present to the second month. Medulloblasts may also be observed in the end leaf of the cornu ammonis and among the small pyramidal cells for two months. Long oval nuclei without tails and others with tails, the neuroblasts, are found here at the same time. The second cortical layer harbors medulloblasts from birth to two years. They appear to be equally distributed in all lobes of the brains of infants in the first year. During the first two

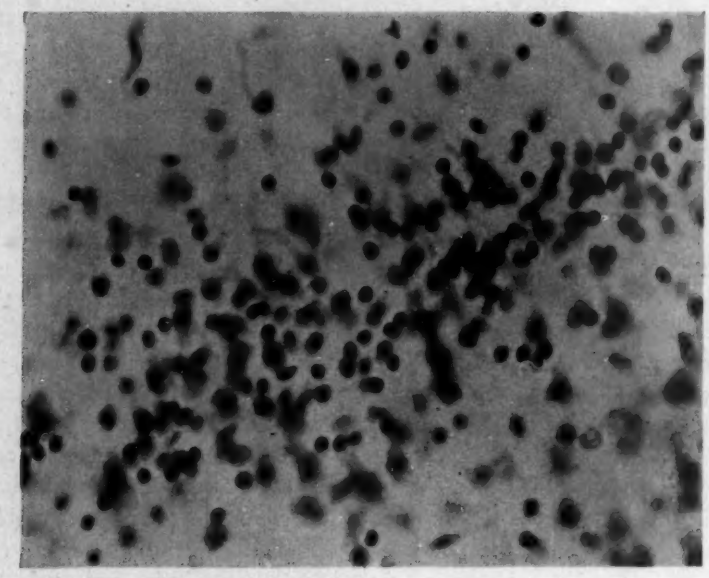

Fig. 1.—The small round nuclei in the second layer are those of medulloblasts, while the larger ones are those of the ganglion cells and astrocytes, at the first month.

postpartum months, they line the rim of the second layer sometimes to a depth of two or three cells, as may be seen in figure 1. In other cases they form a sparsely cellular, although distinct, border. This variation is apparently not related to disease or age. Present also, among the medulloblasts, are very young pyramidal cells and neuroblasts, while spongioblasts and very young astrocytes are not observed. During the next two months, the medulloblasts are less numerous, and the immature ganglion cells are few. Beginning with the fifth month, the medulloblasts are not easily recognized since they are farther apart and are similar to the young oligodendroglia cells, which have increased in number in the second layer as in the other parts of the cerebrum.

The medulloblasts continue to decrease in number during the remainder of the first year although they are demonstrable in all cases to the end of the twelfth month. Young ganglion cells are also rare but may be found for two years. If the medulloblasts survive in the second year they are generally confined to the second layer in a single, although variable, area. Medulloblasts cannot be detected in the other cortical layers with certainty even at birth, since neither group nor row forma-

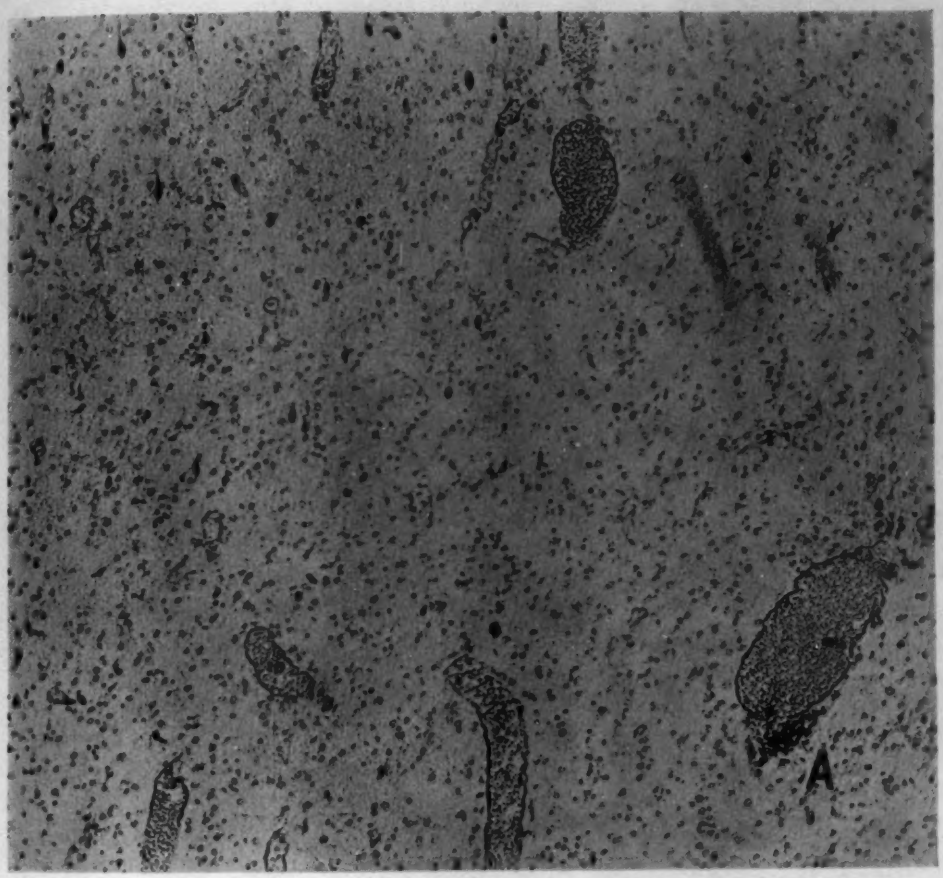


Fig. 2.—At *A* is a perivascular focus of medulloblasts in the upper part of the centrum ovale of the first month.

tion occurs. However, the young pyramidal cells of the first six months suggest that some of them were undifferentiated cells in the first month.

*Centrum Ovale.*—In the upper part of the centrum ovale of the occipital and frontal poles at birth may be found small groups of medulloblasts about the vessels or within the perivascular spaces, as seen in figure 2. They persist in the latter pole for three months but in the

former for only a few weeks. Radiating from the groups are nuclei of various sizes, without cytoplasm, and a few dark oval nuclei with tails, some of which are attached to the vessels. Medulloblasts occur regularly in the lower part of the centrum ovale in all lobes except the temporal, where they appear to be a part of the subependymal groups. They disappear from this part of the occipital lobe in the first month and from the central and frontal regions in the third month. Large sheets of them are found in some cases, as well as heavy bands enclosing entire vessels; generally the groups are much smaller. Nuclear division is infrequent but more prevalent in the cells outside the perivascular spaces. Immature cells are also present near the medulloblasts and decrease in number with them in the second month. Both arterioles and venules, varying in size from large terminating ones to quite small vessels, harbor the medulloblasts.

*Periventricular Recesses.*—Small groups of medulloblasts may be seen on the border of the third ventricle to five months. Between the nucleus caudatus and the ependyma is a heavy layer of medulloblasts at birth. They thin out during the next five months, until none remain. In the recess between the head of the nucleus caudatus and the anterior horn of the lateral ventricle at birth are numerous large groups of medulloblasts near the vessels and in the perivascular spaces. They gradually decrease in number with age but are found in all cases to nine months. Between this nucleus and the thalamus are similar groups that diminish during the first four months, yet may be seen in some cases at nine months. The medulloblasts are less numerous along the posterior horn of the lateral ventricle and do not persist beyond four months. The inferior horn of the lateral ventricle shows a variable number of them in the first month. They may be in large groups and fill most of the space below the ependyma as in figure 3. They usually disappear in five months, but may persist in the inferior angle to the thirteenth month. Little change is observed in the size of the medulloblasts of the recesses at any time. The surrounding astrocytes are fairly mature at birth and numerous, although crowded together. As the brain increases in size, they spread over a greater area, while only oligodendroglia cells appear to be added, either by development from the medulloblasts or by cell division.

*Basal Ganglions.*—All the brains at the first month showed some perivascular medulloblasts in the head of the nucleus caudatus. In the brain of an infant aged 11 days they crossed the entire nucleus in the space of a vessel. In the brains of 2 infants aged 14 and 17 days there were groups of medulloblasts in the spaces of vessels at the edge of the putamen and in the internal capsule. The size and shape of the medulloblasts in these groups appeared to be fairly constant, nor were

there oval nuclei with tails. In the vicinity of the parolfactory area and ependyma are also small round foci of medulloblasts at birth. They persist to seven months but in one case were still present at eighteen months. Small and large, loosely grouped formations of medulloblasts occur around the vessels in the claustrum to four months and at various ages to the end of the second year, which figure 4 shows. Some cellular development takes place among the medulloblasts here, as the oval nuclei of neuroblasts and spongioblasts may be observed.

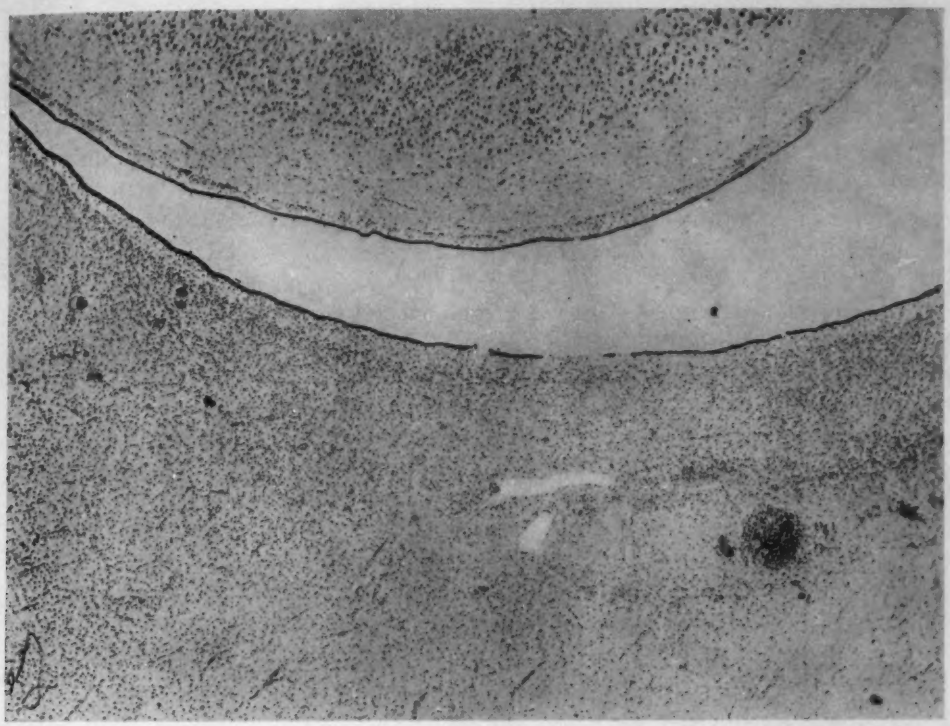

Fig. 3.—Groups of medulloblasts along the inferior horn of the lateral ventricle in the first month.

*Cerebellum.*—Medulloblasts form the outer rim of the marginal layer, while the inner part is composed of rather long oval nuclei with tails. Both parts are found in all cases to nine months and occasionally to twelve months. The layer shows variable thickness not only at birth but at any age of its persistence.

The few cases in which the medulloblasts had not entirely disappeared by the end of the first and second year were: one of whooping cough and convulsions in an infant aged 11 months, in which medulloblasts were present in the outer marginal layer of the cerebellum, the

second cortical layer and the inferior angle of the lateral ventricle; one of acute meningitis in an infant aged 12 months, in which the cells were found in the second cortical layer, the claustrum, the inferior horn of the lateral ventricle and the outer part of the cerebellar marginal layer; one of bronchopneumonia in a child aged 14 months, in which the cells were observed in the second cortical layer and claustrum; one of whooping cough and bronchopneumonia in an infant aged 18 months, in which the cells were present in the claustrum, the second layer of the temporofrontal area and near the parolfactory area; one of osteomyelitis in a child aged 2 years, in which large groups of medulloblasts occurred in the second layer of the area frontoinsularis and in the claustrum. The last are shown in figure 4.

## COMMENT

The time of the disappearance of the medulloblasts from the periventricular areas observed in this examination of infant brains corresponds to the ages given by Roback and Scherer.[4] They made no mention, however, of the foci of medulloblasts in the inferior horn of the lateral ventricle, which are the last to go. The persistence of the cerebral marginal layer through the first postpartum month was also noted by the aforenamed authors, but only in the sulci, whereas it may be noted in the crest, especially in the temporal lobe. They likewise discovered, with Stevenson and Eichlin,[5] that the cerebellar marginal layer may be retained for one year. Yet in neither the paper of Roback and Scherer nor that of Stevenson and Eichlin is there any record of an observation of the medulloblasts present at the same time in the cerebrum. In the 2 cases in my series in which the cerebellar marginal layer was observed at eleven and twelve months, the observation of the medulloblasts of the cerebrum included foci in the periventricular recesses. The significance of the late persistence of the medulloblasts in the latter areas and in the cerebellar marginal layer is unknown, but since the cells are found in these places in cases of megalocephaly, it is possible that the brain enlarges as long as the medulloblasts survive.

The astrocytes in the upper cortical areas are fairly mature at birth and would not, therefore, appear to be the cause of the continued presence of the medulloblasts. Very young pyramidal cells and neuroblasts are, on the contrary, prominent in the second layer during the first two months, when the medulloblasts are most numerous. It is thus reasonable to consider the medulloblasts as the origin of the neuroblasts. Moreover, Filiminoff[6] and Aldama[7] observed young ganglion cells in the second layer during the first year, the time when medulloblasts are

5. Stevenson, L., and Eichlin, F.: Arch. Neurol. & Psychiat. **31**:93, 1934.
6. Filiminoff, I. N.: J. f. Psychol. u. Neurol. **39**:322, 1929.
7. Aldama, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **130**:532, 1930.

likewise present. Medulloblasts of the second layer are doubtless the cells sought after by Aldama, who was not able to establish the source which he believed must supply the young ganglion cells over so long a period. As the function of the second layer ganglion cell has not

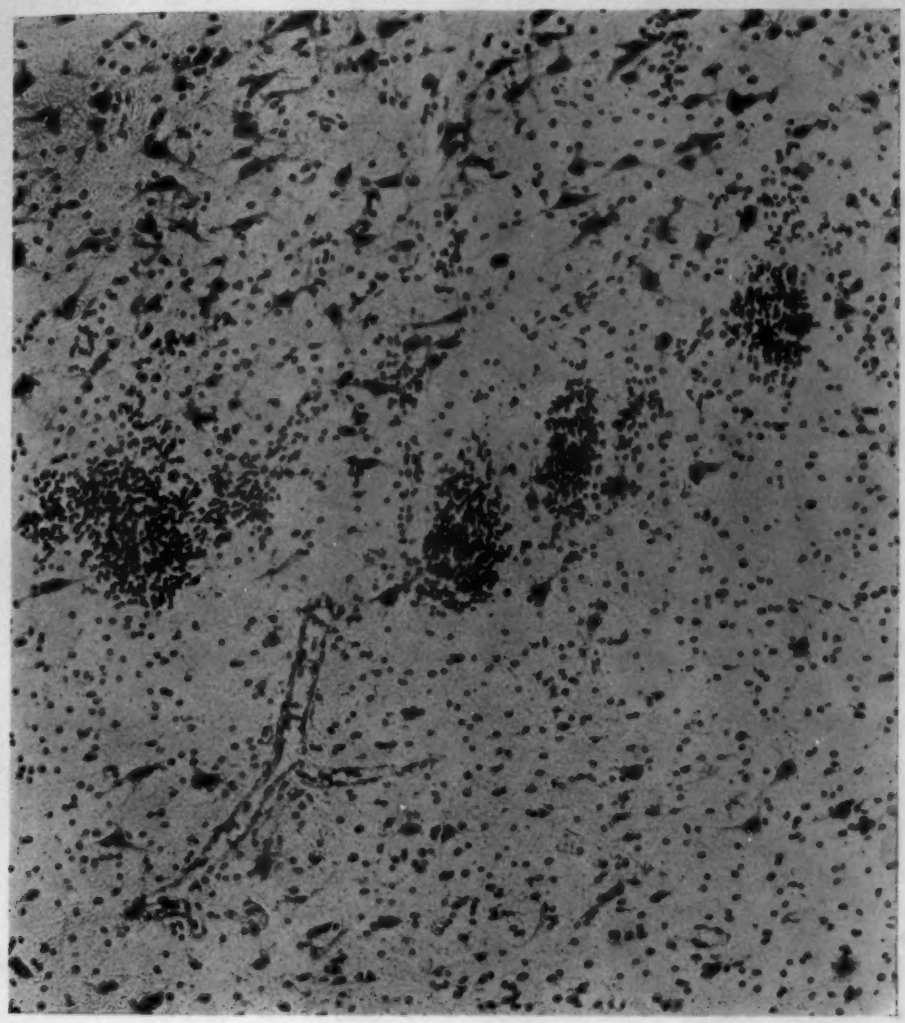

Fig. 4.—Medulloblasts in the claustrum at two years.

been determined, the effect of a large number of medulloblasts here at birth and of their survival into the second year is unfortunately not calculable. The same is true of the medulloblasts in the claustrum and in the parolfactory area.

Although Bielschowsky [8] thought the areas of abnormal cells in the lower and the upper part of the centrum ovale in tuberous sclerosis could be followed down to the ependyma and the cells thus connected with the migrating spongioblasts from this layer, it is evident that the abnormal cells may arise in the foci of medulloblasts of the centrum ovale. This corresponds to the belief [9] that the subependymal tumors in this disease arise from similar foci in the periventricular recesses. Ostertag [9] also regarded the foci of these recesses as important in estimating the position of other brain tumors related to them.

The presence of medulloblasts in the perivascular spaces seems to indicate that they move from one part of the cerebrum to another by way of these spaces, helped by the propulsion of the blood stream. Some confirmation is given this by the vessels of the fetal brain, whose zigzag course may be followed by noting the surrounding medulloblasts. Further support is offered by the attachment of spongioblasts to vessels all over the centrum ovale, since this seems to imply that the transformation of medulloblasts into spongioblasts has taken place near the vessels. Although it is generally believed that spongioblasts migrate from the ependyma,[10] it is apparent that if they do so after birth they must pass through the nucleus caudatus and the lower part of the centrum ovale. The spongioblasts of the latter are most numerous near the foci of medulloblasts, while they are absent in the nucleus caudatus, where the astrocytes are chiefly mature at birth. Furthermore, cellular development in the periventricular recesses after birth has not been found, an observation made earlier by Roback and Scherer.[4] The immature cells of the nucleus caudatus, as well as those of the neighboring internal capsule and putamen, are the medulloblasts, which may be found in the perivascular spaces. This clearly indicated rise of the medulloblasts through the basal ganglions along the vessels adds weight, also, to the assumption that the latter are the means by which the medulloblasts reach the other parts of the brain from the ventricle.

It is evident that maturity comes slowly to some of the brains and that others are quite immature at birth. However, it is not possible to indicate whether consequences attend either incomplete or prolonged cerebral growth.

## SUMMARY

In a study of 46 infant brains it has been found that medulloblasts occur in the cerebral marginal layer and in the first cortical layer during

8. Bielschowsky, M.: J. f. Psychol. u. Neurol. **30**:167, 1923.

9. Ostertag, B.: Einteilung und Charakterische der Hirngewächse, Jena, Gustav Fischer, 1936.

10. Penfield, W.: Cytology and Cellular Pathology of the Nervous System. New York, Paul B. Hoeber, Inc., 1932, vol. 2.

the first month, in the second cortical layer and claustrum to two years and in the parolfactory area to eighteen months. At various times during the first year they disappear from the periventricular recesses, remaining longest in the angle of the inferior horn of the lateral ventricle. The retention of the medulloblasts in the outer rim of the cerebellar marginal layer to eleven and twelve months is accompanied by the presence of similar cells in the cerebrum. The persistence of medulloblasts in the perivascular spaces of the vessels in the nucleus caudatus, the internal capsule and the edge of the putamen the first weeks and in the upper and lower parts of the centrum ovale two months suggests that the medulloblasts travel along the perivascular spaces and are developed in their vicinity.

The significance of the medulloblasts is indeterminate, since so little is known of the function of the cells in the parts of the brain in which they are retained.

# CYTOLOGIC CHANGES IN THE SKIN OF MICE DURING APPLICATION OF CARCINOGENIC AGENTS

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The discovery by Kennaway [1] and by Cook and his associates [2] that a synthetic organic chemical (1,2,5,6-dibenzanthracene) caused formation of malignant tumors in otherwise normal animals gave new impetus to experimental research on cancer. Since that time many carcinogenic chemicals have been discovered, of which the most potent are cholanthrene, methylcholanthrene, 3,4-benzpyrene and 1,2,5,6-dibenzanthracene.

The modus operandi by which these carcinogenic agents effect in cells changes leading to the development of true malignant neoplasms is at present incompletely known. Knowledge of their action may throw some light on the origin of spontaneous malignancy in cells. The response of a normal cell to the application of a specific carcinogenic agent may be similar to the intracellular changes which occur in the body during the transition of normal cells to malignant cells.

I searched the reports of others and conducted an investigation on mice to determine what changes occurred in the size of the nuclei and nucleoli of cells of the skin at different stages during the application of methylcholanthrene and cholanthrene.[3]

## REVIEW OF THE LITERATURE ON CARCINOGENESIS BY CHEMICAL AGENTS

Levine and Bergmann [4] placed the tips of onion roots in a solution of tap water to which had been added benzene containing 1,2,5,6-dibenzanthracene. The roots lost in turgidity within twenty-four hours and failed to grow. Observations

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This work was done at the Institute of Experimental Medicine.

1. Kennaway, E. L.: Biochem. J. **24**:497, 1930.

2. Cook, J. W.; Hieger, I.; Kennaway, E. L., and Mayneord, W. V.: Proc. Roy. Soc., London, s. B. **111**:455, 1932.

3. The cholanthrene used in the investigation was supplied by Dr. L. F. Fieser, of Harvard University. The methylcholanthrene was obtained from the Eastman Kodak Company.

4. Levine, M., and Bergmann, H.: Am. J. Cancer **26**:291, 1936.

by Reimann and Hammett[5] on the developmental growth of a marine hydroid (Obelia geniculata) in sea water cultures showed that 1,2,5,6-dibenzanthracene increased the proliferative activity of the organism by increasing the number of cells. Reuterwall[6] studied tumors produced by tar and 1,2,5,6-dibenzanthracene and concluded that an allergic irritation seemed to be the first link in a long chain of events in the production of malignant tumors.

Tissue cultures have been used as a medium for investigation of carcinogenic chemicals. Hearne[7] grew mouse fibroblasts in a culture medium containing methylcholanthrene. After three days of such growth the cultures contained a high percentage of abnormal mitotic figures and chromosome pairing, and chiasma formation had been induced. Lewis[8] added carcinogenic chemicals (1,2,5,6-dibenzanthracene, 1,2-benzpyrene or methylcholanthrene) to cultures of chick embryo; the cells acquired photosensivity to the electric light used for their observation, and this was often accompanied by an inhibition of cell division, with subsequent mitotic anomalies duplicating many of the types seen in malignant growths.

Tissue metabolism has been found to be altered by 1,2,5,6-dibenzanthracene.[9] This carcinogenic agent when injected into mice produced a change in the animals so that in vitro administration of this substance to slices of the liver brought about a definite disturbance in the oxidation and anaerobic glycolysis of this organ. In the same concentration, 1,2,5,6-dibenzanthracene had no influence on the metabolism of liver from normal, untreated control animals. Pourbaix[10] found that 1,2,5,6-dibenzanthracene, even in minute amounts, in general depressed the respiratory activity of liver from normal guinea pigs 5 weeks of age; respiratory activity was measured in a Warburg apparatus.

Beltrami[11] found a general toxic reaction in the livers and spleens of mice painted with 1,2-benzpyrene, benzene or anthracene, but he concluded that the reaction was not related to the cancer-stimulating activity of the drug. Dunning, Curtis and Bullock[12] injected varying doses of 1,2,5,6-dibenzanthracene and 1,2-benzpyrene into one or more sites in mice and rats. They concluded that the potency for malignancy must be a universal characteristic of cells and that the histogenesis of the tumors was determined by the fortuitous exposure of the various types of cells to the irritant.

Orr[13] injected solutions of phenol red intraperitoneally into mice in the course of experimental carcinogenesis with tar, with 1,2,5,6-dibenzanthracene and with 1,2-benzpyrene. The earliest change was an increase in intensity of staining in the area treated with the carcinogens—the color remained red but was a deeper shade than that of the normal skin. At a later stage foci in the treated skin stained yellow instead of red, and tumors began to appear at the same time. Orr suggested that the yellow coloration was an indication of localized ischemia. In

5. Reimann, S. P., and Hammett, F. S.: Am. J. Cancer **23**:343, 1935.
6. Reuterwall, O.: Ztschr. f. Krebsforsch. **42**:117, 1935.
7. Hearne, E. M.: Nature, London **138**:291, 1936.
8. Lewis, M. R.: Am. J. Cancer **25**:305, 1935.
9. Boström, G.: Acta radiol. **16**:224, 1935.
10. Pourbaix, Y.: Compt. rend. Soc. de biol. **112**:1222, 1933.
11. Beltrami, W.: Tumori **9**:537, 1935.
12. Dunning, W. F.; Curtis, M. R., and Bullock, F. D.: Am. J. Cancer **28**:681, 1936.
13. Orr, J. W.: J. Path. & Bact. **44**:19, 1937.

control observations with noncarcinogenic irritants and spontaneous lesions it was found that yellow staining might occur around healing ulcers.

Cook[14] regarded the term "chronic irritation" as entirely inadequate to describe the effect of applying a carcinogenic substance to animal tissues because in industry many cutaneous diseases are brought about by chemical irritation, but few of the irritants lead to development of malignant tumors. Not one of the carcinogenic hydrocarbons has any apparent irritating effect on human skin, and many hydrocarbons which have chemical and physical properties similar to those of the carcinogenic compounds have no cancer-producing activity whatever.

The relation of an enzyme system to carcinogenic agents was investigated by Boyland.[15] Many polycyclic hydrocarbons are oxidized photochemically in air to products which are able to destroy dehydrogenating enzymes. On the whole, carcinogenic hydrocarbons, such as 1,2,5,6-dibenzanthracene and 1,2-benzpyrene, after oxidation are capable of greater inhibition of lactic dehydrogenase than are the noncarcinogenic hydrocarbons. If the dehydrogenating enzymes in the tissues were destroyed, there would be a tendency to local accumulation of lactic acid. Tumor cells survive and grow in mediums containing lactic acid and are probably less affected by it than are normal cells. If a malignant cell were to appear in tissue in which dehydrogenases were destroyed, it would be at a great advantage and would grow at the expense of normal cells.

Using the spectroscope, Berenblum and Kendal[16] determined the rate of disappearance of 1,2,5,6-dibenzanthracene in aqueous colloidal solution and in lard when injected intraperitoneally into mice. In the excreta of these animals, 1,2,5,6-dibenzanthracene could not be detected, and it was concluded that it must be metabolized in the body into a form no longer possessing the characteristic fluorescent bands.

Domagk,[17] working with mice, found no early change in the skin painted with 1,2-benzpyrene except an alteration in the nucleocytoplasmic ratio and a certain irregularity in the arrangement of the epithelial cells. Wolbach[18] painted a solution of 3,4-benzpyrene on the skin of the backs of mice. It proved to be a destructive agent, and reparative processes were constantly maintained to repair the damage to individual cells. After some degree of resistance to the chemical was acquired by the cells, there occurred a period of hyperplasia. The hyperplasia was to be explained presumably by the reparative responses. Even after the stage of hyperplasia had been reached, the injurious effect on the cells continued and the reparative responses maintained in regions of hyperplasia seemed to be the processes preceding the development of true tumor. There was no evidence that the carcinogenic chemical employed owed its carcinogenic activity to direct stimulation of cell growth.

### ROLE OF NUCLEUS AND NUCLEOLUS

Every living cell contains a nucleus and usually one or more nucleoli. The nucleus is an integral part of the cell and has to do with the metabolism and reproduction of the cell. The functions of the nucleolus remain for the most part obscure.

14. Cook, J. W.: Proc. Roy. Soc., London, s.B. **113**:273, 1933.
15. Boyland, E.: Biochem. J. **27**:791, 1933.
16. Berenblum, I., and Kendal, L. P.: Biochem. J. **30**:429, 1936.
17. Domagk, G.: Ztschr. f. Krebsforsch. **44**:160, 1936.
18. Wolbach, S. B.: Am. J. Path. **13**:662, 1937.

Some investigators consider the nucleolus as the real center of life of the cell while another group [19] of biologists consider that the nucleolus is a waste product of nuclear metabolism and is not necessary for the life of the cell.[20] Saguchi [21] regarded the nucleolus as the biologic center of the nucleus, which is necessary for the formation of the chromatin substance.

The role of the nucleolus during mitosis is obscure. Investigations by Dearing [22] disclosed that the nucleolus may play a part in inheritance, since it is a constantly localized portion of a specific chromosome which is perpetuated by mitosis from cell generation to cell generation.

The fact that nuclei and nucleoli in malignant cells are larger than those in nonmalignant cells of the same tissue is well established.[23] The nuclei of secreting cells are larger than those of nonsecreting cells as was demonstrated by Schairer [24] in the lactating and the nonlactating breast. Ehrich [25] demonstrated that as organs increase their functional activity the nuclei in the active cells grow larger, that there is a geometric increase in the size of the nucleus in malignant cells and that in the cells of benign tumors the nuclei increase in size by swelling. The nuclei of the cells of tar tumors, of cancroids and of tarred skin that was not neoplastic were measured by Epantschin.[26] He found that in all of these conditions the nuclei were larger than the nuclei in epithelial cells of normal skin. The nuclei of the carcinomatous lesions had increased most markedly in size. Ludford [27] regarded the size of the nucleolus as an indication of the degree of metabolism in the cell; the greater the metabolic activity, the larger the total volume of nucleolar material.

In the present state of knowledge the function of the nucleolus seems to be twofold. It takes an important part in the intracellular metabolism, and it is the factory for the production of chromatin

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19. von Derschau, M.: Arch. f. Zellforsch. **12**:220, 1914. Jörgensen, M.: ibid. **10**:1, 1913.

20. Häcker, V.: Arch. f. mikr. Anat. **41**:452, 1893. Heidenhain, M., cited by von Haam and Alexander.[23f]

21. Saguchi, S., cited by von Haam and Alexander.[23f]

22. Dearing, W. H., Jr.: J. Morphol. **56**:157, 1934.

23 (*a*) Quensel, U.: Acta. med. Scandinav. **68**:458, 1928. (*b*) Karp, H.: Ztschr. f. Krebsforsch. **36**:579, 1932. (*c*) MacCarty, W. C.; Haumeder, E., and Berkson, J.: Proc. Staff Meet., Mayo Clin. **8**:38, 1933. (*d*) Page, R. C., and MacCarty, W. C.: Arch. Path. **24**:1, 1937. (*e*) Arndt, G.: Ztschr. f. Krebsforsch. **41**:393, 1935. (*f*) von Haam, E., and Alexander, H. G.: J. Clin. Path. **6**:394, 1936.

24. Schairer, E.: Ztschr. f. Krebsforsch. **43**:1, 1935.

25. Ehrich, W.: Ztschr. f. Krebsforsch. **44**:308, 1936.

26. Epantschin, W.: Ztschr. f. Krebsforsch, **26**:439, 1927-1928.

27. Ludford, R. J.: J. Roy Micr. Soc., 1922, p. 114.

material. Investigations to determine the reasons for, and the significance of, the increase in nucleolar volume in malignant cells should prove to be a fruitful venture in solving the enigma of cancer.

## METHOD OF STUDY

A strain of white mice was used that had a very low incidence of spontaneous malignancy. When malignant lesions developed in this strain, they were usually carcinoma of the breast and rarely lymphosarcoma. Animals aged from 3 to 4 months were used. From 8 to 10 drops of a 0.3 per cent solution of methylcholanthrene in chemically pure benzene was applied to the back of each mouse of a large group three times each week. To another group a 0.3 per cent solution of cholanthrene in benzene was applied. As a control, chemically pure benzene was applied to another group of mice. Immediately after each application of the solutions of benzene, methylcholanthrene and cholanthrene, the animals were exposed to the draft of a small power blower so that evaporation of the benzene would be hastened. Otherwise acute benzene intoxication developed in many animals. Mice were killed at definite intervals in each group until a malignant lesion appeared in one member of the group. Thereafter each mouse remaining in the group was killed as a lesion appeared that was clinically malignant or when the animal appeared to be sick. Immediately after the death of the mouse a piece of skin was removed from its back and fixed in a 10 per cent solution of formaldehyde. Paraffin sections stained with hematoxylin and eosin were used mainly for the study, but some formaldehyde-fixed frozen sections stained with polychrome methylene blue were studied also. The size of the nuclei and of the nucleoli was found to be approximately the same in both types of sections.

Individual cells in the basal layer of the epithelium were studied. In the sections of carcinoma characteristic cells, each with a single nucleolus, were examined so that errors in determination of area would be minimized. Twenty-five nuclei and their contained nucleoli from each section were used for study; an attempt was made to select 5 adjacent cells in as many different fields.

The cells were studied under the oil immersion lens (Bausch and Lomb objective, 1.8 mm.; numeric aperture, 1.25; tube length, 150 mm.; ocular × 15). The shadows of the nuclei and nucleoli were projected through a constant distance onto a paper by means of a camera lucida and were then carefully traced. By use of a stage micrometer this enlargement was found to be 2,400 diameters.

A method for decreasing the steps involved in obtaining the areas of the nuclei and nucleoli was devised. The method, as described in a recent paper by Page and MacCarty,[23d] necessitated the enlarging of nucleoli and the measuring of the area of each nucleus and nucleolus by a planimeter. To avoid this step in all instances, a graph was constructed by plotting on the ordinates the actual areas in square microns of 100 nuclei as determined by the planimeter and on the abscissas the average diameters in millimeters as determined by measuring the longest and the shortest diameters of the same nuclei with an ordinary millimeter rule. A similar graph was constructed for the nucleoli of the same 100 nuclei. After construction of these graphs the average diameter of each nucleus and nucleolus, determined by measuring the longest and the shortest diameter, was obtained in millimeters; then from the graph the area in square microns was read off. Actual measurements of area were not made. The average area of the 25 nuclei and of their nucleoli was calculated, and the mean nuclear-nucleolar ratio was obtained by dividing the average nuclear area by the average nucleolar area.

## OBSERVATIONS

*Normal Skin.*—The normal skin of adult mice varies from 1 to 4 cells in thickness (fig. 1 *A*). There are no definite strata or subdivisions of the epithelium. The free surface has a very small amount of cornified material. There is no well defined stratum germinativum, although the basal layer of epithelial cells serves this purpose. The cells in the basal layer have no definite polarity, and very few of the cells possess visible intercellular bridges. The nuclei of the cells in the basal layer were found to be usually round or oval, and their average area was 31.1 square microns. In 98 per cent of the cells the nucleus contained a single nucleolus. The nucleoli were round or oval, had sharply defined borders and in the majority of nuclei were centrally situated. The average area of the nucleoli was 1.76 square microns. Mitotic figures were not observed in sections of normal skin. Occasional basophilic mast cells were observed in the subcutaneous connective tissue.

*Changes in Skin During Application of a 0.3 per Cent Solution of Methylcholanthrene in Benzene Solution Three Times Weekly to Backs of Animals.*—Two days after the first application of this carcinogenic agent marked change was seen in the skin. In the scattered cells superficial to the basal layer karyorrhexis was occurring. The nuclei were stained lighter and were undergoing vacuolation, while the nucleoli were stained darker and were smaller than normal. The basal cells were more active than normal; the nuclei were increased in size (average area, 45.5 square microns) and were stained more deeply owing to an increase in chromatin content. The average area of the nucleoli in the basal cells had increased to 3.51 square microns, which is more than twice that of normal nuclei (table 1). Nuclei with multiple nucleoli were seen not infrequently, and several mitotic figures were present. There was no evidence of any inflammatory reaction.

Four days after the first application of methylcholanthrene the hair on the back was thinner. The basal cells (fig. 1 *B*) were more active, as shown by further increase in nuclear size (average area, 53.9 square microns) and the nucleoli were much larger (average area, 5.1 square microns) than the nucleoli in the epithelial cells seen two days after the initial application of methylcholanthrene. Occasional spaces were present between adjacent basal cells, and the number of intracellular bridges was increased.

Seven days after the initial application of methylcholanthrene the increase in nucleolar size was most marked (fig. 2 *A*). At this time the average area of nucleoli was 5.8 square microns, or three times that of normal nucleoli, and the numbers of multiple nucleoli in the nuclei were increased (fig. 2 *B*). In scattered areas there was an increase in

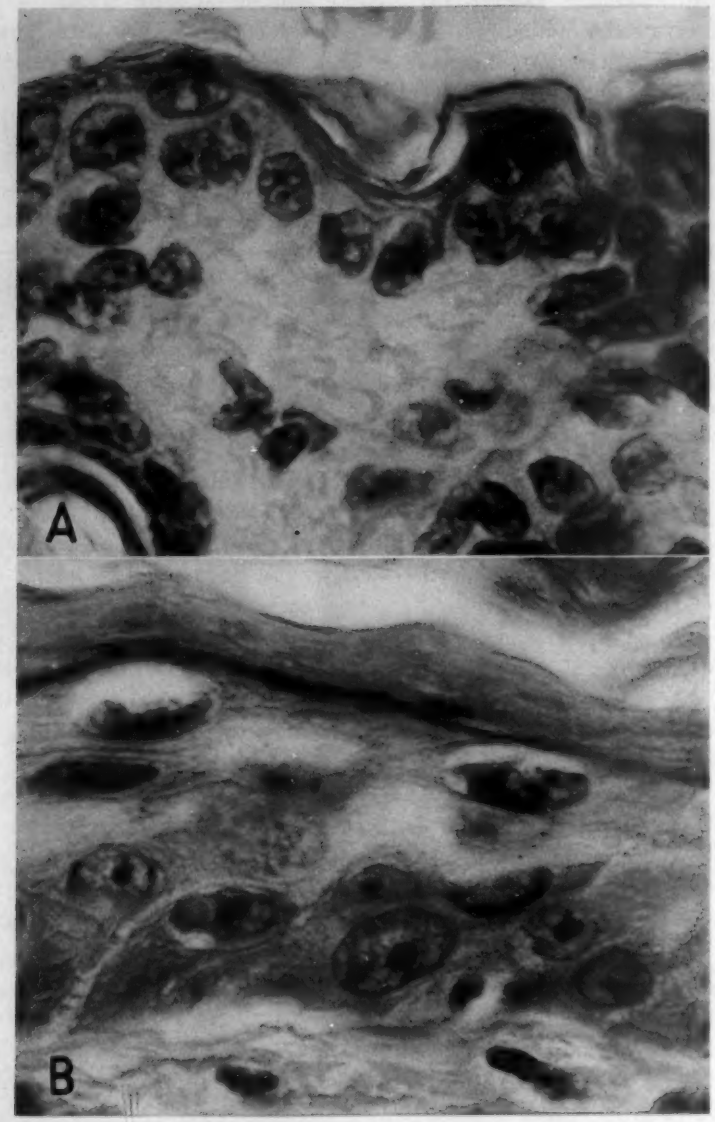


Fig. 1.—*A*, normal skin of a mouse, showing small nucleoli (hematoxylin and eosin; × 1,160). *B*, skin of a mouse four days after application of methylcholanthrene, showing increased size of nuclei and markedly increased size of nucleoli and an increased amount of cornified material on the surface (hematoxylin and eosin; × 1,160).

the thickness of the skin up to from 8 to 12 cells. The basal cells showed definite polarity, with the long axis perpendicular to the surface, in the thickest portions of the skin. The period from the first week to the appearance of the first epitheliomas at the seventh week was characterized by marked hyperplasia of the epithelium. Multiple verrucae (warts) appeared on the skin after the third week. The hair follicles underwent hyperplasia during the first month; then regression occurred. An increase in the amount of subcutaneous connective tissue was accompanied by an increase in mast cells. The size of the nucleoli during this period regressed somewhat from that attained with the primary stimula-

TABLE 1.—*Results of Application of a 0.3 Per Cent Solution of Methylcholanthrene in Benzene Three Times a Week to the Backs of Mice*

| Days After First Application | Animals | Mean Nuclear-Nucleolar Ratio | Average Area, Square Microns | |
|---|---|---|---|---|
| | | | Nuclei | Nucleoli |
| | 10* | 17.6:1 | 31.1 | 1.76 |
| 2 | 4 | 12.9:1 | 45.5 | 3.51 |
| 4 | 4 | 10.5:1 | 53.9 | 5.1 |
| 6 | 3 | 10.7:1 | 53.8 | 5 |
| 7 | 2 | 8.8:1 | 52 | 5.8 |
| 14 | 4 | 10.1:1 | 40.5 | 4.01 |
| 21 | 3 | 9.7:1 | 36.9 | 3.8 |
| 28 | 2 | 10:1 | 36.9 | 3.7 |
| 35 | 2 | 9.44:1 | 34 | 3.6 |
| 49 | 3† | 15.3:1 | 45.9 | 3 |
| 63 | 3† | 12.6:1 | 49.1 | 3.9 |
| 68 | 1 | 10.4:1 | 40.5 | 3.87 |
| 70 | 1† | 8.5:1 | 51.25 | 6 |
| 72 | 1† | 10.6:1 | 32 | 4.9 |
| 74 | 1 | 10.1:1 | 47.5 | 4.7 |
| 76 | 1† | 10.1:1 | 48.5 | 4.8 |
| 77 | 1 | 11.8:1 | 49 | 4.13 |
| 80 | 2 | 12.1:1 | 40 | 3.3 |
| 90 | 3 | 13.1:1 | 39.3 | 3 |
| 98 | 2† | 11.6:1 | 52.4 | 4.5 |
| 103 | 1 | 11.7:1 | 43 | 3.65 |
| 115 | 3† | 10.9:1 | 45.8 | 4.2 |
| 136 | 3† | 11.2:1 | 52.6 | 4.7 |

* These were normal animals for controls.
† These animals had squamous cell epithelioma of the skin of the back.

tion in the first week and an average area of between 3 and 4 square microns was maintained. The nuclei also were smaller than they had been during the first week and their area was from 34 to 40 square microns.

The first carcinomas (squamous cell epitheliomas) occurred after seven weeks' application of methylcholanthrene. From this time up to the end of the experiment at nineteen and a half weeks a malignant lesion developed in each of 17 animals (65.4 per cent) and failed to develop in 9 (34.6 per cent). The tumors were diagnosed and graded under the direction of Dr. A. C. Broders (table 2). Of the 17 carcinomas, 3 were of grade 1, 10 of grade 2 and 4 of grade 3 (fig. 3 *A*). The average nuclear and nucleolar areas in the carcinomas were greater than those in the hyperplastic epithelium of the animals in which malig-

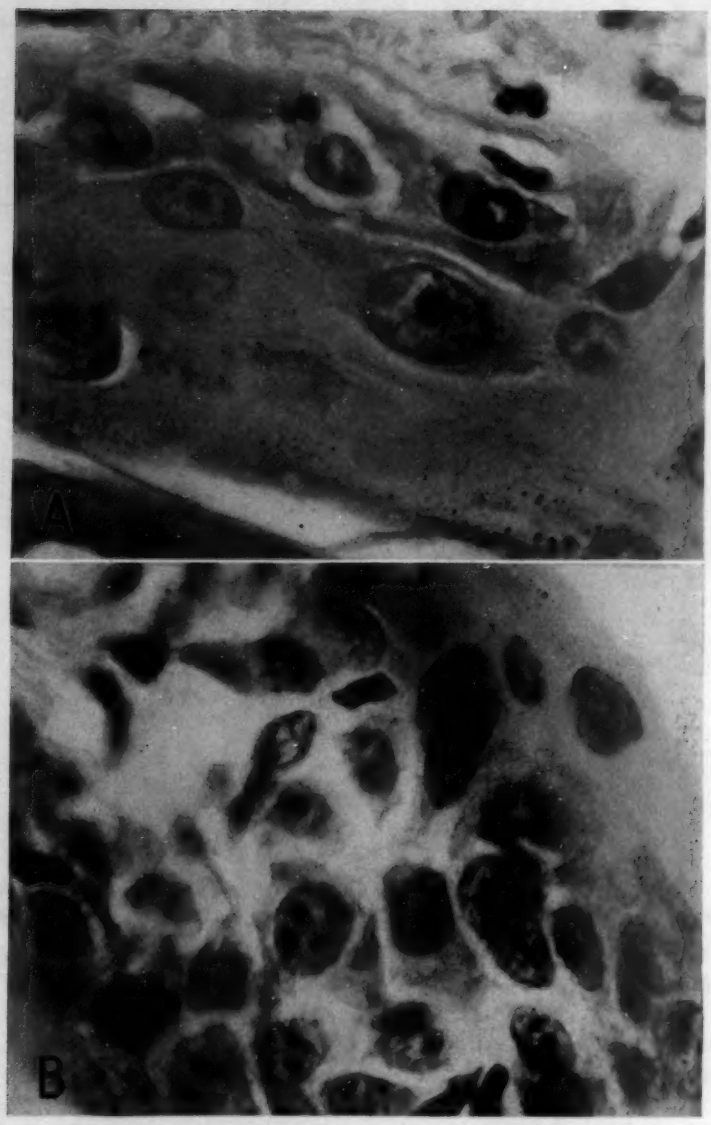


Fig. 2.—*A*, skin of a mouse seven days after application of methylcholanthrene, showing large nucleoli (hematoxylin and eosin; × 1,160). *B*, skin of a mouse eleven days after application of methylcholanthrene, showing multiple nucleoli (hematoxylin and eosin; × 1,160).

nancy did not develop. The largest nucleoli, which had an average area of 6 square microns, were observed in a squamous cell epithelioma ten weeks after the initial exposure to methylcholanthrene (fig. 3 *B*). One animal had dual malignant lesions, a squamous cell epithelioma and an adenocarcinoma, developing in close proximity.

*Changes in Skin During Application of a 0.3 Per Cent Solution of Cholanthrene in Benzene Three Times Weekly to Backs of Animals.*—The cytologic changes occurring in the epithelium after application of cholanthrene were of the same nature, although not as great as those in animals to which methylcholanthrene was applied. The nuclei and nucleoli were immediately stimulated by cholanthrene to an increase in size (table 3), and this increase in size was maintained throughout the experiment. The first carcinoma (squamous cell epithelioma) occurred after eighteen weeks' application of cholanthrene. From the

TABLE 2.—*Results of Application of a 0.3 Per Cent Solution of Methylcholanthrene to Mice for from Seven to Nineteen Weeks*

| Grade of Malignancy | Average Days From First Application* | Animals | Mean Nuclear-Nucleolar Ratio | Av. Area, Square Microns | |
|---|---|---|---|---|---|
| | | | | Nuclei | Nucleoli |
| 1 | 72 | 3 | 10.6:1 | 44.3 | 4.18 |
| 2 | 96 | 10 | 12.1:1 | 49.6 | 4.10 |
| 3 | 79 | 4 | 10.5:1 | 50.4 | 4.8 |
| Animals with hyperplasia (warts) only | 99 | 9 | 12:1 | 41.3 | 3.44 |

* Of the animals which received applications for seven weeks or more, 65.4 per cent had malignant growth and 34.6 per cent had no evidence of it.

eighteenth week to the end of the experiment, at the twenty-eighth week, malignant growth developed in 9 animals (69 per cent); 4 (31 per cent) had only hyperplasia of the epithelium (table 4). Six of the carcinomas were of grade 2. Three were of grade 3.

The nucleoli in the carcinomas produced by application of cholanthrene were larger than those in the carcinomas of corresponding grade of malignancy produced by methylcholanthrene. In the animal on which the experiment continued for twenty-three weeks, a fibrosarcoma, grade 1, was present in the painted area and was surrounded by hyperplastic epithelium without evidence of malignant change.

*Changes in Skin During Application of Benzene Three Times Weekly to Backs of Animals.*—A control group of 32 mice was painted with benzene to determine if the medium in which the carcinogenic chemicals were applied would in itself cause cytologic change in the skin. The nuclei varied in size from 28.7 to 37.5 square microns while the nucleoli, which varied from 1.2 to 1.67 square microns (table 5), were slightly smaller than the nucleoli in normal animals. The varia-

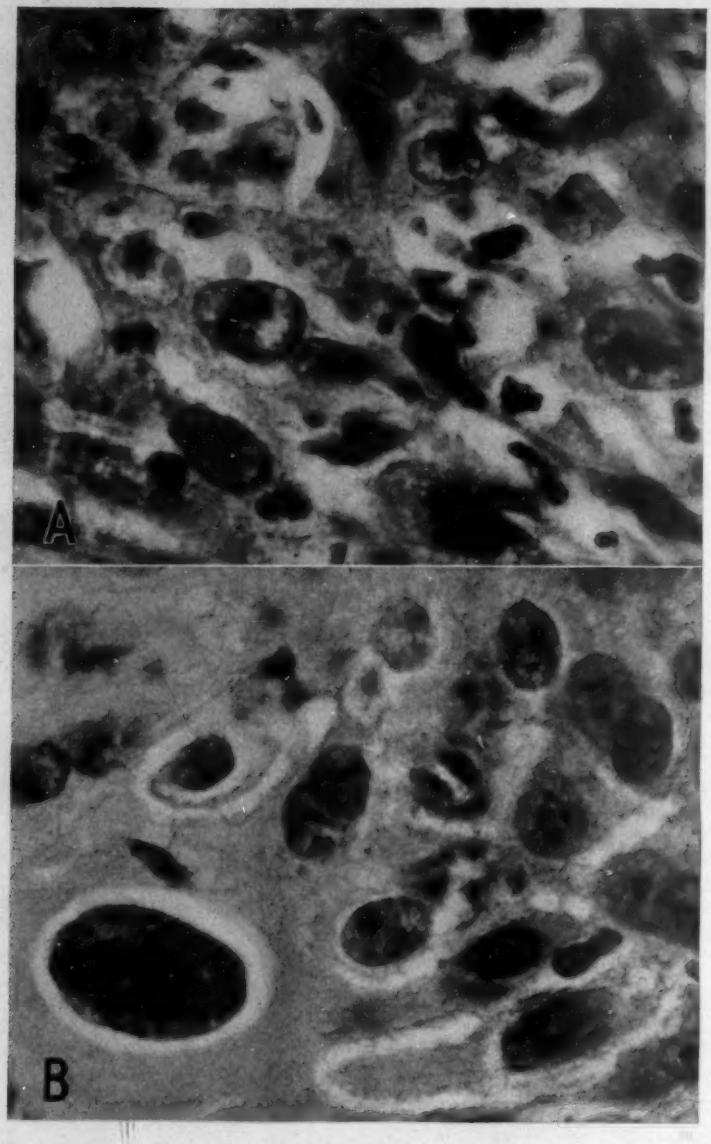


Fig. 3.—*A*, squamous cell epithelioma, grade 3, one hundred and four days after first application of methylcholanthrene. The cells show a marked degree of dedifferentiation. Some nuclei contain large single and multiple nucleoli (hematoxylin and eosin; × 1,160). *B*, squamous cell epithelioma, grade 1 (carcinoma in situ), seventy days after first application of methylcholanthrene. The cells contain large nucleoli; there is a giant nucleus with three nucleoli (hematoxylin and eosin; × 1,160).

TABLE 3.—*Results of Application of a 0.3 Per cent Solution of Cholanthrene in Benzene Three Times a Week to the Backs of a Group of Mice*

| Days After First Application | Animals | Mean Nuclear-Nucleolar Ratio | Average Area, Square Microns | |
|---|---|---|---|---|
| | | | Nuclei | Nucleoli |
| | 10* | 17.6:1 | 31.1 | 1.76 |
| 2 | 4 | 14.8:1 | 28.6 | 2.6 |
| 4 | 4 | 12.2:1 | 45.2 | 3.7 |
| 7 | 2 | 12.6:1 | 46.6 | 3.7 |
| 14 | 3 | 10.2:1 | 38 | 3.7 |
| 28 | 3 | 12.5:1 | 36.3 | 2.9 |
| 48 | 2 | 11:1 | 42.9 | 3.9 |
| 65 | 2 | 11.9:1 | 36.9 | 3.1 |
| 83 | 1 | 12.1:1 | 38.7 | 3.2 |
| 124 | 1† | 10.5:1 | 48.3 | 4.6 |
| 144 | 1 | 12:1 | 42 | 3.5 |
| 144 | 2† | 9.4:1 | 43.6 | 4.65 |
| 161 | 1 | 15.99:1 | 35 | 2.2 |
| 164 | 2† | 11.2:1 | 43.7 | 3.9 |
| 182 | 2† | 10.4:1 | 55.1 | 5.3 |
| 182 | 1 | 18:1 | 36 | 2 |
| 196 | 1 | 17.7:1 | 39 | 2.2 |
| 196 | 2† | 11.4:1 | 53.6 | 4.7 |

* These were normal animals for controls.
† These animals had squamous cell epithelioma.

TABLE 4.—*Results in Animals of Application of a 0.3 Per Cent Solution of Cholanthrene in Benzene for from Eighteen to Twenty-Eight Weeks*

| Grade of Malignancy* | Average Days From First Application | Animals | Mean Nuclear-Nucleolar Ratio | Av. Area, Square Microns | |
|---|---|---|---|---|---|
| | | | | Nuclei | Nucleoli |
| 2 | 168 | 6 | 10.2:1 | 46.9 | 4.6 |
| 3 | 163 | 3 | 10.4:1 | 52.6 | 5.06 |
| Animals with hyperplasia (warts) only | 171 | 4 | 16 | 38.4 | 2.4 |

* Of the animals which received applications for three months or more, 69 per cent had malignant growth and 31 per cent had no evidence of it.

TABLE 5.—*Results in Control Group to Which Benzene Was Applied Three Times a Week*

| Days After First Application | Animals | Mean Nuclear-Nucleolar Ratio | Average Area, Square Microns | |
|---|---|---|---|---|
| | | | Nuclei | Nucleoli |
| | 10* | 17.6:1 | 31.1 | 1.76 |
| 2 | 4 | 21.17:1 | 30.7 | 1.45 |
| 4 | 4 | 23.1:1 | 34.7 | 1.5 |
| 6 | 4 | 22.6:1 | 36.2 | 1.6 |
| 10 | 2 | 22.4:1 | 37.5 | 1.67 |
| 14 | 4 | 19.1:1 | 28.7 | 1.5 |
| 28 | 3 | 23.1:1 | 30 | 1.3 |
| 56 | 3 | 24.1:1 | 28.9 | 1.2 |
| 84 | 2 | 25.2:1 | 33.5 | 1.33 |
| 112 | 3 | 22.3:1 | 30.3 | 1.36 |
| 140 | 3 | 20.7:1 | 31.1 | 1.5 |

* These were normal animals for controls.

tions in size of nuclei and nucleoli throughout the time that the benzene was applied corresponded so closely to normal variations that it was apparent that no significant cytologic change in the skin was caused by the benzene.

## COMMENT

The carcinogenic chemicals (cholanthrene and methylcholanthrene) used in this study have an early and definite effect on the intracellular structures. There is a toxic effect, as evidenced by the dead cells. The skin attempts to protect itself by causing an increased amount of cornified material to be laid down on the surface to which the chemical agent is repeatedly applied. There is no evidence that an inflammatory reaction plays any part in the carcinogenic action of these chemicals.

The sequence of events in the nucleoli of epithelial cells following application of cholanthrene or methylcholanthrene was an immediate increase in size, which reached a maximum of from two to three times normal in one week; then there was a slight decrease in size from the maximum, but the nucleoli remained about twice normal size until a malignant lesion occurred; next the nucleoli again increased in size. Two peaks in nucleolar size occurred, the first one week after the first application and the other at the time the lesion became malignant. The nuclei responded to cholanthrene and methylcholanthrene by an immediate enlargement to one and two-thirds normal size, which was maintained until malignancy occurred, when the nuclei were again increased in size. The size of the epithelial cells was increased throughout the period of application of the carcinogenic agents. The size of the nucleoli in the malignant lesions was only about a third larger than that of the nucleoli in the skins that failed to produce any malignant change.

One of the actions of cholanthrene and methylcholanthrene on the cells of the skin appears to be that of direct stimulation, as evidenced by an increase in the size of the cell, the nucleus and the nucleolus.

## SUMMARY

A simplified method for obtaining the area of nuclei and nucleoli is described. Cholanthrene or methylcholanthrene in 0.3 per cent solution in benzene was applied three times weekly to the backs of mice. A control group was used in which benzene alone was applied.

The carcinogenic agents caused an immediate increase in the size of the cell, nucleus and nucleolus, which was most marked in the nucleolus.

In the group to which methylcholanthrene was applied, squamous cell carcinoma appeared within seven weeks and in that to which cholanthrene was applied, it appeared within eighteen weeks. In both

groups, that to which cholanthrene was applied and that to which methylcholanthrene was applied, malignant lesions of grades 1, 2 and 3 occurred. The majority of the lesions were of grade 2.

In the group to which benzene was applied no changes were observed in the nuclei or in the nucleoli.

One of the actions of cholanthrene and of methylcholanthrene appears to be a direct stimulating effect on the nucleus and nucleolus.

# LATENT PERIOD IN GROWTH OF SPONTANEOUS MAMMARY CARCINOMA IN FEMALE MICE OF THE A STRAIN

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Several recent investigations have emphasized that the initial period of the origin and growth of spontaneous carcinoma of the mammary gland, before the carcinoma can be seen or palpated, should, if possible, be determined. For example, it has been shown that there is a precocious drop in the hemoglobin of those mice which belong to strains that are susceptible to carcinoma as compared with those belonging to strains that are more resistant to cancer.[1] The question naturally arises, Does this drop precede the onset of malignancy or does it occur contemporaneously with it? An investigation dealing with the determination of differences in electric potential in mice susceptible to cancer disclosed that with the growth of cancer there may be a disturbance (within certain age groups) of bioelectric patterns within the organism.[2] The time relation between this probable disturbance and the actual inception of cancer should be carefully investigated.

It has been shown further that atypical nodules (early described by Ehrlich, Apolant, Haaland and others) are more frequent in the mammary glands of mice which belong to strains highly susceptible to cancer.[3] It is a well established fact that even if from forty-five to fifty of these nodules are present in a mammary gland a few only, perhaps not more than five or six, ever become neoplastic. Recent evidence has shown that these hyperplastic nodules may have persisted within the

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Mr. G. C. Bittner made the statistical analysis of the data presented.

From the Department of Anatomy, Yale University School of Medicine.

This experiment has been made possible by grants from the International Cancer Research Foundation, the Anna Fuller Fund and the Research Funds of the Yale University School of Medicine.

1. Strong, L. C., and Werner, T. H.: Am. J. Cancer **26**:767, 1936; **27**:115, 1936. Strong, L. C.: ibid. **27**:500, 1936. Strong, L. C., and Francis, L. D.: Arch. Path. **23**:202, 1937. Francis, L. D., and Strong, L. C.: Unpublished data.

2. Burr, H. S.; Smith, G. M., and Strong, L. C.: Bio-Electric Properties of Cancer-Resistant and Cancer-Susceptible Mice, Am. J. Cancer, to be published.

3. Gardner, W. U.; Strong, L. C., and Smith, G. M.: Unpublished data. Haaland: Scientific Report on the Investigations of The Imperial Cancer Research Fund, London, 1911, vol. 4, p. 30. Woglom, W. H.: Studies in Cancer, New York, Columbia University Press, 1913, p. 205.

mammary gland for as long as five months before progressively growing cancer is detected.

The present investigation deals with a statistical analysis of the growth rates of spontaneous mammary carcinomas in 100 female mice of strain A.[4]

## PROCEDURE

As soon as the tumors were palpable, the mice were separated into individual cages and kept on a control diet of oatmeal. Water was given twice daily in a small glass container. Extreme caution was employed throughout in order to permit all mice to live as long as possible. The boxes were sterilized twice a week; the cotton bedding was changed every day; the mice were inspected every day and periodically dusted with tobacco dust or bathed with an insecticide in order to control all surface parasites.

TABLE 1.—*Growth of Mammary Carcinoma as Determined at Weekly Intervals in One Hundred Female Mice of Strain A*

| Weeks | Tumors Measured | Average Diameter, Cm. |
|---|---|---|
| Start | 100 | 0.90 |
| 1 | 93 | 1.16 |
| 2 | 93 | 1.34 |
| 3 | 83 | 1.49 |
| 4 | 80 | 1.71 |
| 5 | 69 | 1.94 |
| 6 | 52 | 2.07 |
| 7 | 54 | 2.28 |
| 8 | 43 | 2.35 |
| 9 | 39 | 2.52 |
| 10 | 30 | 2.56 |
| 11 | 24 | 2.79 |
| 12 | 21 | 2.80 |

Body weights were taken once a week. The tumors were measured by the vernier scale once a week in the three dimensions. Since the shape of the average tumor remains uniform (within limits, however) the longest linear dimension is a function of the actual bulk of the tumor. An accurate determination of the actual bulk of the tumor is impossible owing to some cyst formation, necrosis in the later stages of growth and hemorrhage into the tumor. It is felt that with the use of 100 mice the role of individual fluctuations is reduced to a minimum and that the analysis of the average growth rate of the tumors is a relatively true index of the phenomenon under investigation.

It is realized that even with the present data obtained on 100 carefully controlled mice with spontaneous tumors errors of sampling are not entirely eliminated. The time required, however, for working with spontaneous tumors in mice necessitates the use of smaller numbers than seem essential for ideal statistical analysis.

## OBSERVATIONS

The measurements of the longest linear dimensions of the tumors at weekly intervals are averaged in table 1 and presented graphically in chart 1 (solid line).

4. Strong, L. C.: J. Hered. **27**:21, 1936.

The number of tumors measured in the successive weekly periods diminished owing to the fact that some of the mice had died in the interim. The average length of life for strain A mice with spontaneous tumors was found to be fifty-five and two-tenths days. Death was due to several factors as follows: (1) the actual size and position of the spontaneous tumor, (2) the presence of metastases in the lungs, (3) congestion in the lung, (4) hemorrhage from the surface of the tumor or (5) intercurrent infections. There is apparently a tendency of the mice with the larger tumors to die earlier, but not all show this tendency (for instance, a mouse with a small primary tumor may have a large number of metastases in the lungs). Mice also differ considerably as to the age at which spontaneous tumors develop. A further detailed analysis of the data, therefore, will be forthcoming when this series of

TABLE 2.—*Values Obtained with the Actual Data on the Growth Rate of Spontaneous Tumors According to the Formula* $y = a + bx + cx^2$

| x | a | bx | $cx^2$ | y |
|---|---|---|---|---|
| —4 | 0.64754 | —0.74325 | +0.05553 | —0.04018 |
| —3 | 0.64754 | —0.49550 | +0.02468 | 0.17672 |
| —2 | 0.64754 | —0.24775 | +0.00617 | 0.40596 |
| —1 | 0.64754 | 0.0 | 0.0 | 0.64754 |
| 0 | 0.64754 | 0.24775 | —0.00617 | 0.88912 |
| 1 | 0.64754 | 0.49550 | —0.02468 | 1.11836 |
| 2 | 0.64754 | 0.74325 | —0.05553 | 1.33526 |
| 3 | 0.64754 | 0.99100 | —0.09872 | 1.53982 |
| 4 | 0.64754 | 1.23875 | —0.15425 | 1.73204 |
| 5 | 0.64754 | 1.48650 | —0.22212 | 1.91192 |
| 6 | 0.64754 | 1.73425 | —0.30233 | 2.07946 |
| 7 | 0.64754 | 1.98200 | —0.39488 | 2.23466 |
| 8 | 0.64754 | 2.22975 | —0.49977 | 2.38752 |
| 9 | 0.64754 | 2.47750 | —0.61700 | 2.50804 |
| 10 | 0.64754 | 2.72525 | —0.74657 | 2.62622 |
| 11 | 0.64754 | 2.97300 | —0.88848 | 2.73206 |
| 12 | 0.64754 | 3.22075 | —1.04723 | 2.82556 |

x = weekly deviation from the starting point, S
$y = a + bx + cx^2$ b = 0.24775
a = 0.64754 c = —0.00617

mice may be enlarged. For the present study, a preliminary statistical analysis of the data is justifiable.

The value of x was taken to be the distance (expressed in weeks) along the horizontal axis. The starting point, S, is equal to 1 in terms of x. The formula used for the analysis of the data was $y = a + bx + cx^2$. The complete values for all components of the equation are given in table 2.

The theoretic curve (dash line) based on the equation $y = a + bx + cx^2$ is given in chart 1. It may be seen that the theoretic curve fits the observed data closely. By extrapolation with minus values of x (distance from the starting point, S), it is obvious that the theoretic curve approaches zero between the values of —3 and —4 (the value of y at —4 was —0.04018).

It is probably true, therefore, that on the average spontaneous tumors exist as growing tumors for approximately from three to four weeks before they can be detected by palpation.

In order to ascertain whether there was any tendency of the rate of growth to approach a sigmoid curve, such as is found especially in normal and embryonic growth, the present determinations were divided into two classes depending on the size of the tumors at the start of the experiment. It was felt that if there was any tendency toward sigmoidism it would show up in that class containing mice with the smallest initial tumors. The curves are presented in charts 2 and 3. There is no tendency toward sigmoidism. All curves obtained are practically identical in shape and, no doubt, the equation $y = a + bx + cx^2$ applies to all of them. Consequently, the tumors that were larger at the start

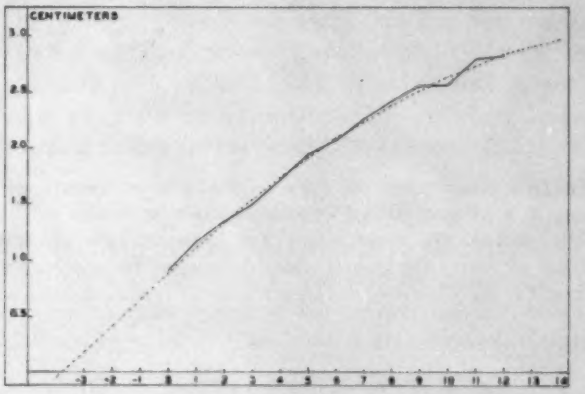


Chart 1.—This chart shows graphically the actual data obtained on the growth of spontaneous tumors (solid line) and the symbolic curve obtained by the formula $y = a + bx + cx^2$ (dash line). The number of weeks after the start ($S$) of the experiment is plotted along the base line; the sizes of the tumors (longest linear dimension in centimeters) are plotted on the vertical line.

of the experiment were larger not because they had grown faster but because they had existed longer within the organism before they were detected.

## COMMENT

The probable disturbance in the bioelectric pattern of the organism (within a restricted age group) took place approximately from one to two weeks before the tumors became palpable.[2] In the present investigation it became clear, therefore, that such a disturbance does not precede the origin of cancer but (if the observed disturbance is significant) may be concomitant with it.

The analysis of the data dealing with the drop in hemoglobin before the onset of malignancy has not reached the point where final conclu-

sions can be drawn. The type of curve (a progressively declining one) starting in early adult life and continuing up to the time of the onset of cancer indicates that this drop precedes the neoplastic process. The time relation within a few weeks of the time at which the tumor becomes palpable must be further analyzed (in view of the present data) in order to ascertain whether the precocious drop in hemolgobin is an

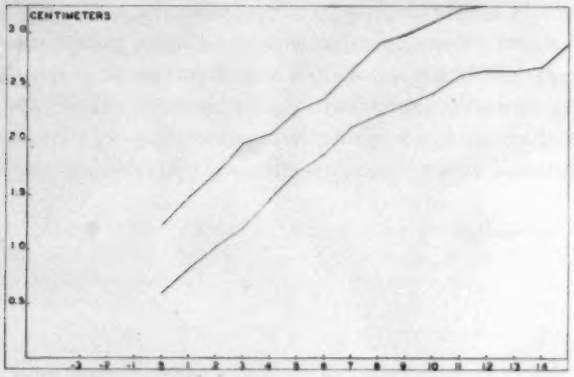


Chart 2.—This chart gives the data on the rate of growth of spontaneous tumors when the mice are divided into two classes according to the size of the tumors at the start of the experiment. The lower curve represents all tumors between 0.2 and 0.9 cm. in the longest linear dimension; the upper curve, all tumors between 1 and 1.7 cm. The two curves of growth remain equidistant from each other.

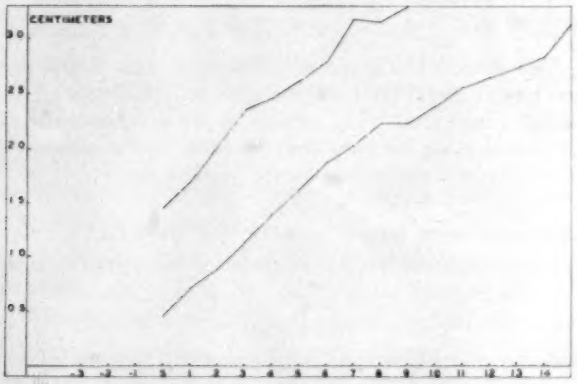


Chart 3.—This chart, like chart 2, gives the data on the rate of growth of spontaneous tumors when the mice are divided into two classes according to the size of the tumors at the start of the experiment. The lower curve represents tumors between 0.2 and 0.7 cm. in longest linear dimension at the start of the experiment; the upper curve, tumors between 1.3 and 1.7 cm. Again as in chart 2, the two curves of growth remain equidistant from each other.

independent constitutional function or one correlated with the onset of cancer.

The analysis of the atypical nodules in the mammary glands of mice susceptible to cancer opens up a new line of thought. Histologic evidence has shown that these hypertrophic nodules may persist (or even undergo internal changes) for as many as one hundred and fifty days before the eventual tumor becomes palpable. Thus it is clear that if the "progressively growing" tumor has been present within the organism only from three to four weeks, as seems evident from the present investigation, some new factor must have been introduced into the equation, perhaps concomitantly with the origin and growth of spontaneous carcinoma of the mammary gland. No analysis of a mathematical formula applicable to the present data could prevent the symbolic curve from approaching zero long before the one hundred and fifty day period is reached. The opinion has been expressed that this new element may be comparable to a somatic mutation. Experimental evidence for this conception has already been published.[5] It is hoped that by the timing of the actual start of cancer as a "progressively growing" nodule further light on the actual nature of neoplastic disease may be forthcoming.

## SUMMARY

The data presented indicate that cancer in the mammary gland of the female mouse of strain A as a "progressively growing" unit exists from three to four weeks before it can be detected and that the precocious drop in the hemoglobin precedes the actual onset of carcinoma of the mammary gland.

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5. Strong, L. C.: Genetics **10**:294, 1926; J. Exper. Med. **4**:713, 1926; J. Cancer Research **13**:103, 1929. Bittner, J. J.: Papers Michigan Acad. Sc. Arts & Lett. **11**:349, 1930; Am. J. Cancer **15**:2202, 1931. Cloudman, A. M.: Am. J. Cancer **16**:568, 1932; Genetics **17**:468, 1932.

# CARDIOVASCULAR AND OTHER LESIONS IN CALVES FED DIETS LOW IN MAGNESIUM

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Until recent years the literature on the gross and microscopic pathologic alterations associated with disturbances in the metabolism of magnesium has been rather meager. As a matter of fact, Peters and Van Slyke,[1] in their interpretations of quantitative clinical chemistry, published in 1931, stated that "thus far no clinical significance has been attached to changes in magnesium metabolism."

Hirschfelder[2] found that oral administration of as much as 3 Gm. of magnesium sulfate per kilogram of body weight to rabbits suffering from mercuric chloride injury to the tubules of the kidneys would cause a rise in the plasma magnesium sufficient to produce coma.

Greenberg and his co-workers[3] reported marked degenerative changes particularly in the kidney and to some extent in the heart in rats on diets poor in magnesium. The changes in the heart consisted of myocardial degeneration and polyblastic infiltration. In the kidney there were multiple casts and widespread tubular degeneration with fibrosis and polyblastic infiltration.

Schrader and his co-workers,[4] working with rats on diets low in magnesium, noted mild degenerative changes in the tubules of the kidneys. There were also some changes in the skin and in the liver.

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Published by permission of the Director of the Experiment Station as Journal Article No. 314 N. S.

From the Sections of Dairy Husbandry and Animal Pathology, Michigan Agricultural Experiment Station.

1. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931, vol. 1, p. 862.

2. Hirschfelder, A. D.: J. Biol. Chem. **104**:647, 1934.

3. Greenberg, D. M.; Anderson, C. E., and Tufts, E. V.: J. Biol. Chem. **114**: xliii, 1936.

4. Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition **14**:97, 1937.

In the liver there was mild foaminess of the cellular elements, vascular proliferation and perivascular accumulation of small round cells. Cramer [5] found that diets low in magnesium produced in rats extensive degenerative lesions in the renal glomeruli and tubules. Brookfield [6] noted changes in the liver and injury to the glomeruli and necrosis of the tubular epithelium.

In 1935 Duncan, Huffman and Robinson [7] reported that a failure to raise calves to maturity on a ration of supplemented whole milk was associated with low plasma magnesium. With plasma magnesium low, the calves exhibited tetany and other symptoms which were not distinguishable from the tetany and other symptoms of calves with low plasma calcium.

We had the opportunity of observing these calves as they came to autopsy. The calves all showed a definite pathologic picture, which was different from that usually seen in calves. There were plaques in the endocardium and intima of the heart and blood vessels, but these were at first not recognized as calcified lesions. Later work [8] has shown that the symptoms and lesions can be prevented by adding magnesium compounds to the diet. With these changes that were associated with a deficiency in mind, it was decided to make a histologic examination of the tissues of calves which came to autopsy with a history of low plasma magnesium. In a previous preliminary report,[9] before the tissues had been stained for calcium, the lesions were described as "basophilic hyalin-like necrosis of the yellow elastic fibers." At the time this paper was read, it was pointed out that there was probably some calcification of the lesions.

## EXPERIMENTAL PROCEDURE

For more detailed data regarding feeding procedures, symptoms and the values obtained on chemical examination of the blood the two previous papers [10] may be consulted. As a rule the calves were started on their respective rations at the age of 30 days and usually came to autopsy at the age of approximately 6 months. Usually the autopsy was performed within from one to three hours after death. However, often they died at night, so that the autopsy was delayed until the following day.

Blocks of tissue were taken from the calves as they came to autopsy and were at first fixed in Zenker's fluid. In later cases solution of formaldehyde U. S. P. diluted 1:10 was used as a fixative in order to prevent the removal of

5. Cramer, W.: Lancet **2**:174, 1932.

6. Brookfield, R. W.: Brit. M. J. **1**:848, 1934.

7. Duncan, C. W.; Huffman, C. F., and Robinson, C. S.: J. Biol. Chem. **108**:35, 1935.

8. Huffman, C. F., and Duncan, C. W.: J. Dairy Sc. **19**:440, 1936.

9. Moore, L. A.; Sholl, L. B., and Hallman, E. T.: J. Dairy Sc. **19**:441, 1936.

10. Duncan, Huffman and Robinson.[7] Huffman and Duncan.[8]

the calcium. The blocks were embedded in paraffin, sectioned and stained with eosin-alum-hematoxylin stain. Some of the formaldehyde-fixed sections were stained in a 1 per cent silver nitrate solution in sunlight. Some which were stained with silver nitrate were also run through the regular eosin-alum-hematoxylin stains. A few of the formaldehyde-fixed sections were decalcified with nitric acid and stained with eosin-alum-hematoxylin. Some other sections were stained with Mallory's aniline blue, Verhoeff's, Weigert's and Unna's orcein stains.

## MACROSCOPIC OBSERVATIONS

Thirty-eight calves at autopsy showed typical gross lesions, which were apparently confined mainly to the endocardium, the intima of the blood vessels and the surfaces of the diaphragm. There was considerable variation in appearance and size of the lesions in the various cases. If the injury was of a milder type, there was noted only slight roughening of the intima of the aorta and of the pulmonary artery. The lesions were barely visible as slightly elevated light-colored plaques from 1 to 2 mm. in diameter, and circular, oval or rather linear in outline. Larger, more marked lesions were from 2 to 5 mm. in the largest diameter, variable in shape, rough and irregular on the surface and grayish white. In some cases of marked injury the lesions were confluent over considerable areas. Much variation was also noted in the extent of the lesions. In some cases there were only small patches in the endocardium and pulmonary artery. In cases in which the changes were more severe the involvement was much more generalized, one animal showing general involvement of practically all of the larger arteries, with some of the smaller vessels also presenting marked generalized sclerosis. The surfaces of the spleen and diaphragm were also involved to a variable degree, the lesions causing these surfaces to appear much thickened and hardened in some of the cases of severe injury. One animal showed some involvement of the capsule of the thymus. Ten of the animals showed variable degrees of bronchopneumonia, and 17 animals showed variable degrees of nephritis.

Of the 38 calves that came to autopsy, 4 showed lesions in the endocardium of the right ventricle; 25, in that of the left ventricle; 12, in that of the left atrium, and 12, in that of the right atrium; 3 showed lesions of the epicardium; 15 showed lesions in the intima of the pulmonary artery; 5, in that of the carotid artery; 11, in that of the aorta, and 3 in that of the jugular; 13 showed lesions in the capsule of the spleen, and 22 subepicardial hemorrhages on the heart.

## MICROSCOPIC OBSERVATIONS

The principal pathologic change in the various organs mentioned in the gross description was calcification of the yellow elastic fibers. That the process was calcification[11] was shown by a marked increase of total calcium, phosphorus and magnesium as shown by analysis of the ash constituents of the aortas. In those organs in which the elastic fibers were interlaced with muscle or collagenous fibers, the latter were also involved in the calcification. However, it was possible to establish definitely that the calcification took place first in the yellow elastic fibers by studying the internal elastic membrane of some of the small arteries and also the yellow elastic fibers of the aorta.

In sections which were fixed with Zenker's solution and stained with eosin-alum-hematoxylin, the calcified fiber stained more deeply with both eosin and alum-

11. Duncan, C. W.: Unpublished data, Section of Agricultural Chemistry, Michigan Agricultural Experiment Station.

Fig. 1.—Left side of the heart, showing marked calcification of the endocardium; silver nitrate stain.

hematoxylin than the normal fiber. The color was more red than blue, so that it would best be described as bluish red. The color depended to some extent on how thick the blocks had been cut before being placed in Zenker's solution and on the consequential removal of calcium and probably of mordanting substances, such as iron. This was brought out in one block of spleen, which was cut so thick that the calcium in the center of the block was not removed. The calcified areas in the center of the block stained blue-black, while at the periphery of the section they stained bluish red. Fixation in Zenker's solution was not satisfactory because the calcified areas did not stand out in contrast in the stained sections. In formaldehyde-fixed tissue stained with eosin-alum-hematoxylin, the calcified fibers stood out in distinct contrast, being stained blue-black. With this method it was possible to identify the lesions with considerable ease.

Formaldehyde-fixed tissue stained with silver nitrate alone or counterstained with eosin-alum-hematoxylin also proved satisfactory. The calcium was stained black, and where the calcification was heavy, it appeared as a homogeneous mass. Where the calcium deposits were less dense, the individual granules were easily seen.

Since the changes seemed to involve the yellow elastic tissue principally, it was decided to use some of the special elastic stains to see whether some definite injury could not be detected previous to calcification. Verhoeff's, Weigert's, Mallory's aniline blue and Unna's orcein stains were used. Fragmentation and degeneration of the elastic fibers were seen in the aortas and large arteries. The calcified and the normal fibers stained with equal intensity so that there was no contrast. The only way of distinguishing the apparently normal fiber from the calcified fiber was by the loss of the wavy appearance of the latter and its displacement from the normal arrangement. In some sections stained with orcein the calcified areas appeared somewhat lighter, but the contrast was not great.

Formaldehyde-fixed sections which were decalcified on the slide for a few minutes in 5 per cent nitric acid solution gave fair satisfaction. Here the calcified areas stained blue, and there was considerable contrast. Formaldehyde-fixed sections stained with eosin-alum-hematoxylin and sections stained with silver nitrate gave the best slides for studying histologic alterations. A description of the change noted in each particular tissue and of the particular peculiarities follows.

*Endocardium.*—The calcification took place in the elastic fiber network under the subendothelial layer of the endocardium.

The shape of the lesion varied with the length of the time the process had been established. In the more recent lesion calcification appeared as a narrow band in the elastic fiber network, as shown in figure 2 *C*. In this instance there was no elevation of the endothelial or subendothelial layers above the normal level of the endocardium. In older lesions necrotic areas developed in which the calcium appeared as a more or less homogeneous mass, the characteristics of the fiber having been lost altogether. In the centers of these foci there was a reddish-staining material in the sections stained with eosin-alum-hematoxylin, which was probably necrotic débris, the result of decalcification of some of the fibers, as shown in figure 2 *D*. In these areas the endothelial and subendothelial layers were irregularly elevated, although these layers remained intact.

Injury to the elastic fibers previous to calcification did not appear to be present, nor was there evidence of peripheral calcification of the fibers, but the calcification appeared to take place diffusely through the fiber. In some sections the calcification appeared to take place first in the deeper fibers of the elastic layers.

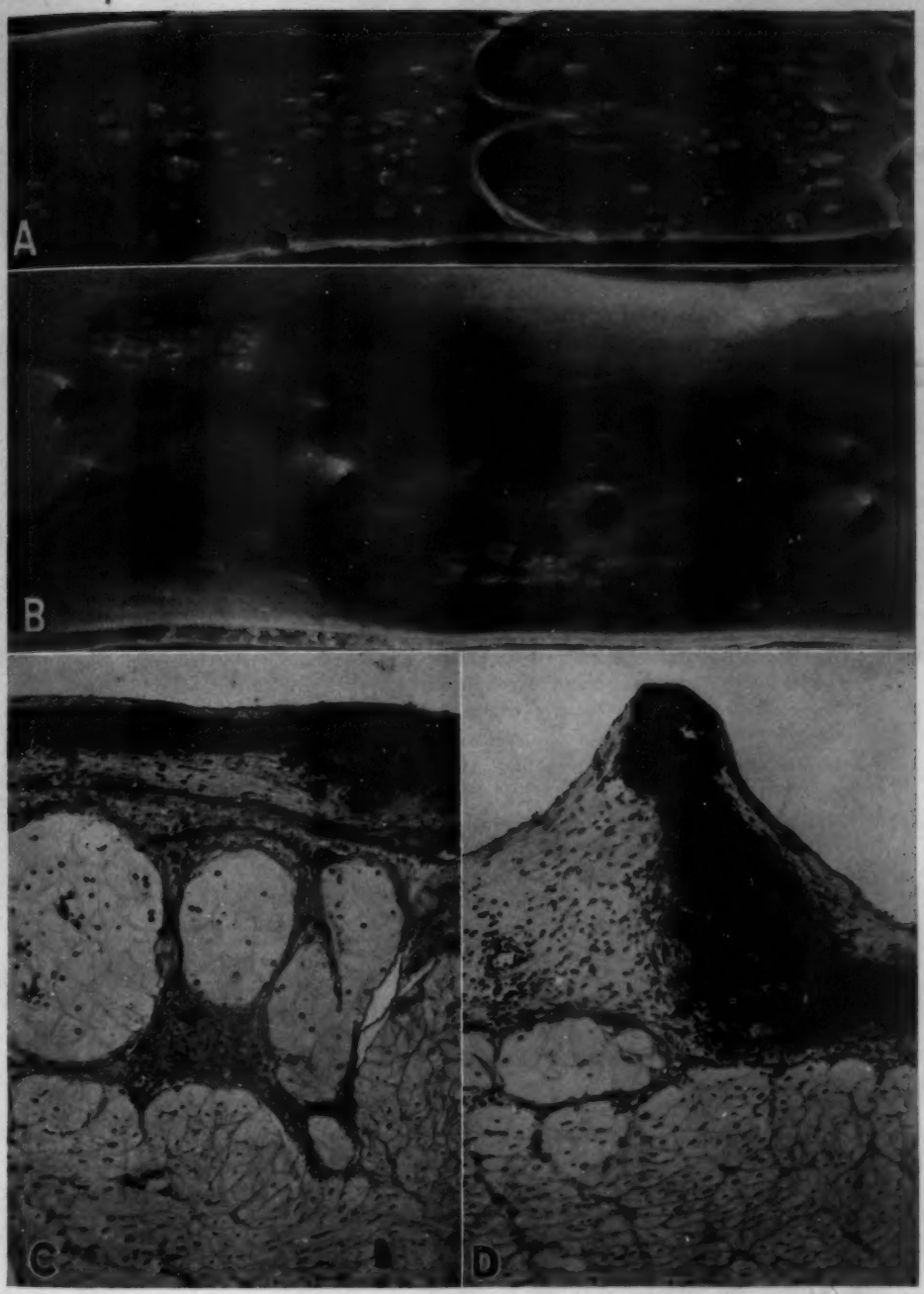


Fig. 2.—*A*, calcification of the jugular vein. *B*, moderate calcification of the aorta. *C*, calcification of the endocardium; eosin-alum-hematoxylin stain; × 120. *D*, calcification of the endocardium, showing elevation; eosin-alum-hematoxylin stain; × 120.

In some sections there was considerable evidence of a fibroblastic reaction around the injured areas of the endocardium, as shown in figure 3 *C*, although the reaction was not common. Lymphocytes in considerable numbers were noted once.

In some sections there appeared to be considerable thickening of the endocardium, with considerable space between the fibers. The exact nature of this condition was not established, although edema was suspected.

*Purkinje Fibers of the Heart.*—Of 10 instances in which sections from the heart included a few Purkinje fibers, 6 showed what appeared to be an extensive degenerative process in these fibers. Evidence of degeneration was suggested by the presence of material varying from very fine granules in some fibers to particles of sufficient size to occupy from one half to two thirds of the cross-sectional area of the fibers, as shown in figure 3 *B*. Usually, however, the fiber contained many smaller granules rather than a single large particle.

In the sections stained with eosin-alum-hematoxylin the granules and particles stained brownish yellow, which reminded one of deposits of blood or lipochrome pigment. The material in the granules and particles was homogeneous in appearance. The condition resembled somewhat that described as brown atrophy of the muscle fibers of the heart.

This condition of the Purkinje fibers was not present in all sections of the same heart. Sections taken from one area might show the degeneration while sections taken from another might not. At the time the tissue was taken the purpose was to study the changes in the endocardium. The location and extent of these degenerative changes in the heart should be studied further.

In 2 cases there was definite calcification of the Purkinje fibers. Here in cross sections the fibers usually contained little cytoplasm and showed a few scattered granules of calcium salts in what appeared to be a lumen at the center of the fiber, as shown in figure 3 *A*. Complete calcification of the entire cross section of the fiber was occasionally seen.

*Myocardium.*—Of 17 animals studied for myocardial changes, 2 showed myocardial hemorrhages, and 2 others showed extensive degeneration and considerable calcification of the myocardial fibers. A third one showed some swelling of the fibers with a staining reaction different from the normal, but there was no calcification.

In the animals showing calcification of the muscle fibers, the first suggestion of a degenerative process was deeper eosin staining with little or no swelling of the fibers in the longitudinal sections. Calcification then appeared to take place first with deposition of very fine granules. This continued till the fiber was blue-black and homogeneous in appearance. The process appeared to take place diffusely within the fiber, as shown in figure 4 *B*. One case was observed in which not all the necrotic fibers were calcified but appeared as vacuoles.

There was no indication of a cellular reaction surrounding the degenerated or calcified areas. It appeared that the changes in the myocardium took place only after considerable injury to the endocardium. It seems possible that the changes are secondary, probably due to changes in the coronary vessels. Calcification of the coronary arteries was noted in one of the sections which showed myocardial changes.

*Arteries of the Elastic Type.*—Throughout the full length of the aorta there were areas of calcification in the intima, media and adventitial layers, as shown in figure 4 *A*, with some variations in location and extent between different animals

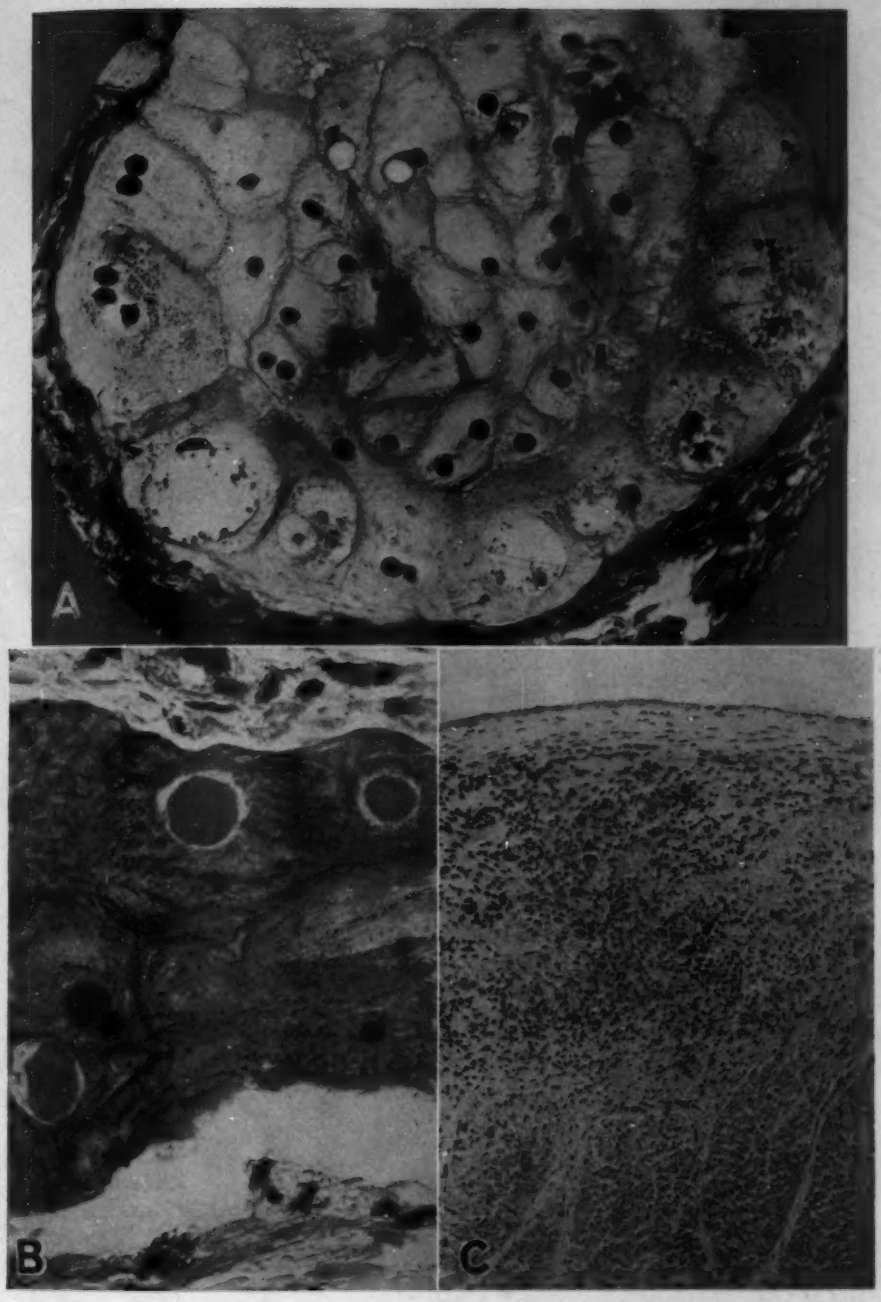


Fig. 3.—*A*, vacuolation and deposition of calcium salts in the Purkinje fibers; eosin-alum-hematoxylin stain; × 350. *B*, degeneration of Purkinje fibers; eosin-alum-hematoxylin stain; × 580. *C*, fibroblastic reaction in the endocardium; eosin-hematoxylin stain; × 120.

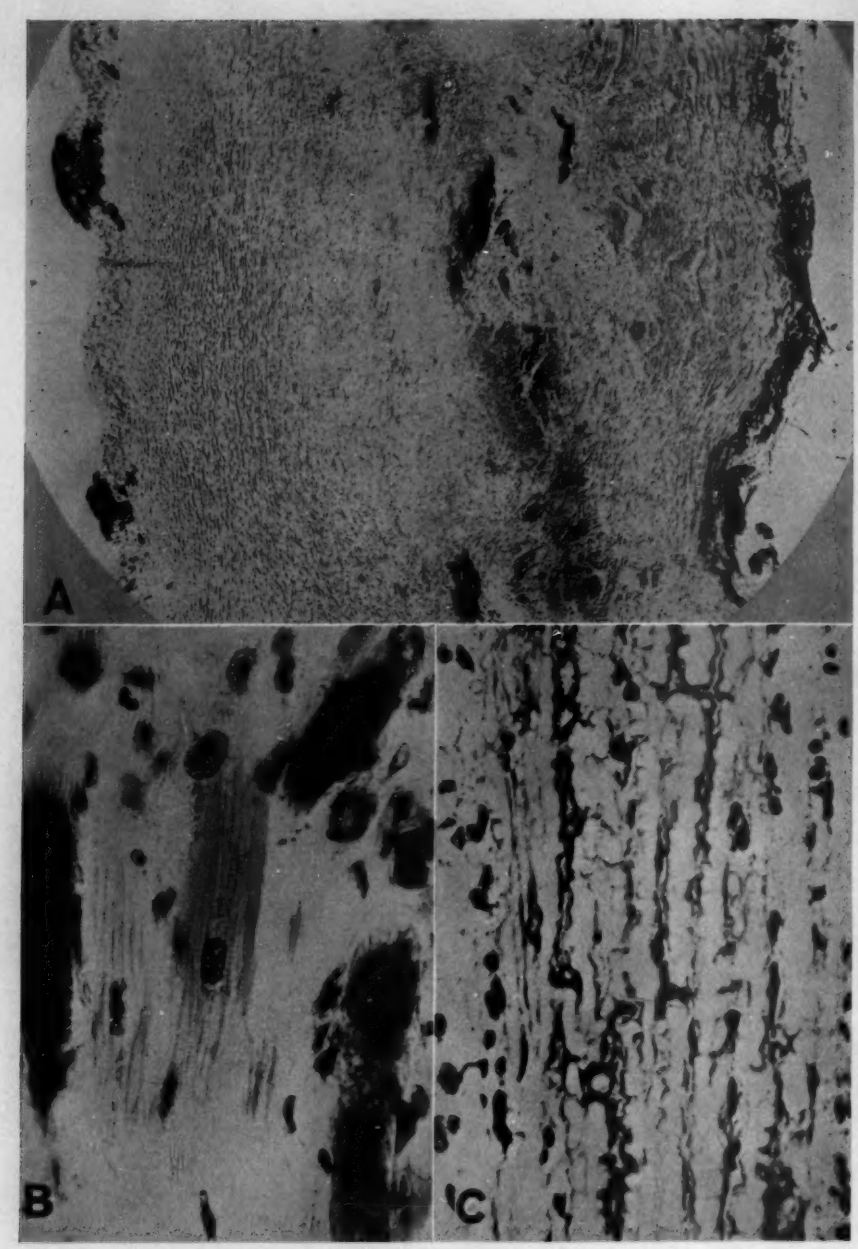


Fig. 4.—*A*, calcification of the intima, media and adventitia of the aorta; silver nitrate stain; × 25. *B*, calcification of the heart muscle; eosin-alum-hematoxylin stain; × 580. *C*, peripheral calcification of yellow elastic fibers of the aorta; eosin-alum-hematoxylin stain; × 580.

studied. The morphologic alterations were identical regardless of the location. The changes consisted of a primary calcification of the elastic elements. These alterations were also present in the pulmonary artery.

The areas of calcification followed the direction of the longitudinal axis of the vessel and therefore appeared linear in shape in the longitudinal sections and varied in width from 1 up to from 8 to 10 fibers. The lesion of the intima did not involve the endothelial or subendothelial layers, and although there was no apparent histologic change in these layers they were elevated through the presence of the lesion in the fenestrated membrane.

In many cases the calcification of the elastic fibers appeared to take place at the periphery of the fibers or in the tissue surrounding the fiber, since the staining appeared darker at the edges of the fibers, as shown in figure 4 *C*. In the areas where the fibers were calcified they lost their wavy appearance and became straightened. In some instances it was possible to distinguish fragmentation of the fiber previous to calcification, while in others calcification appeared to take place without previous alteration of the fiber.

Verhoeff's and Weigert's stains were of no value in demonstrating the lesions where the calcifications had taken place, which might or might not indicate that the fibers were not seriously injured in the calcified areas. In sections stained with orcein, the calcified areas appeared somewhat lighter in color, but the contrast was not great. Some sections showed considerable fragmentation and degeneration in the intima, as shown in figure 5 *B*, with little calcification. The nature of the degeneration was not determined.

In a few sections there was some indication of degeneration of the muscle fibers. This condition was not extensive, however. In some cases there was proliferation of fibroblasts in the intima in the areas of calcification.

*Internal Yellow Elastic Membrane of Arteries of the Muscular Type.*—The smaller arteries, such as would usually be included in blocks of tissue from the heart, spleen and other organs, were not usually involved in this process except possibly in the kidney. The larger arteries, such as those leading from the aorta or the carotid artery, were the ones most usually involved.

The changes in the vessels of the muscular type involved the internal yellow elastic membrane and the adventitia, with no involvement of the media. The change in the intima was most generally limited to the yellow elastic membrane, leaving the endothelial layer intact. On cross section, as shown in figure 5 *A*, there was considerable variation in involvement of the membrane. In the calcified areas the membrane had lost its wavy appearance and had become somewhat straightened, and the calcium appeared almost as a calcified homogeneous band. In these areas the band of calcium was usually from two to three times the width of the original membrane and caused some elevation of the endothelium. Where the calcification was less extensive the individual granules could be seen and the membrane retained somewhat its wavy characteristics. Sometimes indications of fragmentation and slight swelling were seen, but these changes were not marked. Some cellular reaction, fibroblastic in nature, was noted surrounding the areas of calcification in the intima.

*Adventitia of Arteries.*—There was considerable calcification of the adventitial layer of some of the larger arteries of the muscular type, as shown in figure 5 *C*. In the areas of calcification the calcium salts appeared as a homogeneous granular mass in the center, irregular at the edges. In lesions where the calcium was not deposited so heavily the collagenous fibers could be distinguished, and although they contained granular deposits of calcium salts, much less was present than in

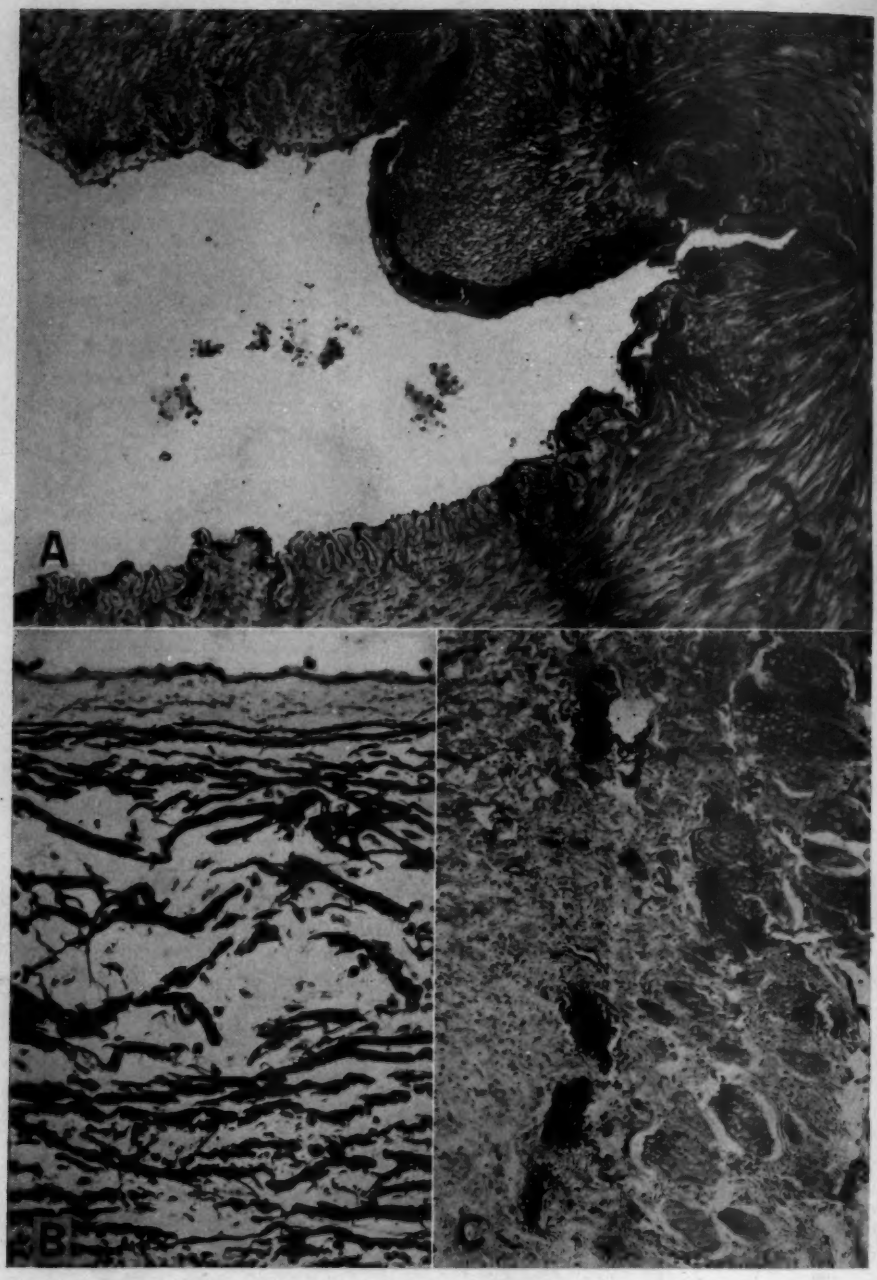


Fig. 5.—*A*, calcification of the intima of an artery, with some proliferation of fibroblasts; × 84. *B*, fragmentation of the yellow elastic fibers of the intima of the aorta, Verhoeff's stain; × 350. *C*, calcification of the adventitia of an artery; eosin-alum-hematoxylin stain; × 84.

the yellow elastic fibers. This indicates that the collagenous fibers were more resistant to the injury and were the last to become involved in the process. Where the injury was less extensive the yellow elastic fibers showed fine granular deposits of calcium and some fragmentation. The collagenous fibers appeared normal where the injury was less extensive. There was no indication of cellular reaction in proximity to the calcified areas.

*Pleural and Peritoneal Surfaces of the Diaphragm.*—The lesions were present on both sides of the diaphragm and presented an identical microscopic appearance. They were usually linear in shape and involved the yellow elastic tissue elements, the principal change being a calcification of this tissue, as shown in figure 6 *B*. As the lesion developed to such an extent as to present a plaque macroscopically, many of the fibers became involved in one lesion so that it became difficult to differentiate each individual calcified fiber. In this case the collagenous tissue was also involved in the calcification.

In the sections which were stained with eosin-alum-hematoxylin or with silver nitrate and eosin-alum-hematoxylin, it was impossible to detect any extensive change in the fibers previous to calcification. In other words the fiber appeared healthy and normal up to the point of calcification. Occasionally, however, some indication of fragmentation was in evidence. No cellular reaction surrounded any of the calcified areas.

In sections stained with Mallory's aniline blue the injured yellow elastic fibers took on a more reddish color as compared with the normal greenish yellow color.

In sections stained with Verhoeff's and Weigert's stains there was no color differentiation between the normal and the calcified yellow elastic fibers.

*Spleen.*—Of 18 cases in which the spleen was saved for histologic examination, 9 showed definite histologic alteration and some of the spleens were extensively involved, as shown in figure 6 *A*. The lesions were present in the capsule and trabeculae, but in either case the histologic changes were identical. In some sections as much as one third of all the elements of the trabeculae were calcified, and most all of the trabeculae were affected.

Where the trabeculae were sectioned longitudinally the lesions were usually linear in shape and occupied the centers of the trabeculae. The calcification followed the longitudinal axis of the fibers of the trabeculae. The length of the lesion was variable, and the width varied from that of a few fibers up to one half to two thirds of the width of some of the trabeculae. The pattern of calcification was not always identical in each case. In some instances the areas were fairly well circumscribed, while in others they appeared to show much branching of the calcifying process.

The calcification was usually heavy in the spleen, and in the sections stained with eosin-alum-hematoxylin it was impossible to distinguish the individual fibers in the centers of the lesions. At the edges, however, it was possible to note the calcification of the individual fibers. It was, however, impossible to establish definitely what tissues were being calcified first—whether yellow elastic, collagenous or muscle fibers. However, from analogy, supposedly the yellow elastic fibers were affected first. As the lesion developed, all three tissues were involved in the calcifying process. Further, it was impossible to distinguish degenerative changes previous to calcification.

In sections stained with Mallory's aniline blue it was impossible to distinguish more definitely the individual fibers of the calcified areas. This indicated the retention of the form and shape of the fibers even after calcification had taken

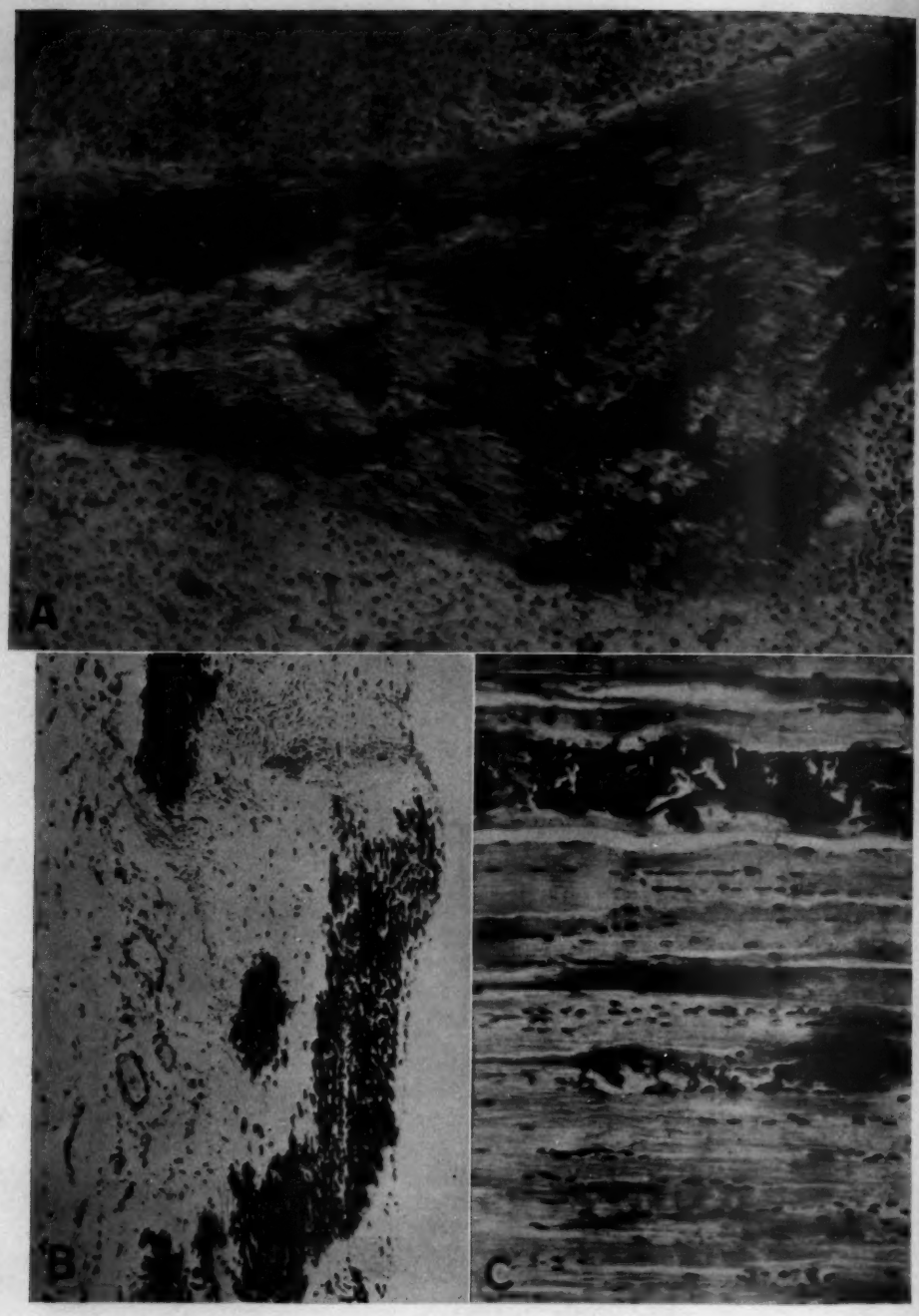


Fig. 6.—*A*, calcification of a trabecula of the spleen; eosin-alum-hematoxylin stain; × 200. *B*, calcification of the peritoneal covering of the diaphragm; eosin-alum-hematoxylin stain; × 120. *C*, degeneration and calcification of skeletal muscle; eosin-alum-hematoxylin stain; × 140.

place. Where there was a definite change in the fibers, as indicated by the stain, the tissue took on a more yellow color.

In sections stained with Weigert's stain the areas where calcification had taken place were indistinct. The yellow elastic fibers stained a slightly lighter shade than where there had been no calcification, indicating that even after calcification the fibers had not completely degenerated or lost their form. Most of the calcium had probably been removed, since this stain contains about 2 per cent hydrochloric acid.

In sections stained with silver nitrate and eosin the calcium deposits appeared as an agglomerate, and it was impossible to distinguish the individual fibers.

*Skeletal Muscle.*—In the histologic study of the surfaces of the diaphragm it was noted that there was calcification of the muscle tissue of the organ, which was not seen in the macroscopic examination. With these changes in mind, muscle tissue was saved from the foreleg and hindleg muscles of a few of the animals as they came to autopsy.

Longitudinal sections of the muscles showed calcification of the fibers when stained with silver nitrate and also when stained with eosin-alum-hematoxylin, as shown in figure 6 *C*. The fibers which were almost entirely calcified often showed a rather corkscrew effect in the section, especially in sections of the diaphragm, probably due to displacement of the calcified fiber during contraction of the normal fibers and its inability to straighten out again. The calcified fibers had usually increased in diameter from two to three times the normal. The calcification usually involved only one fiber in a particular area, but there was very apparent injury, probably mechanical, to the surrounding muscle fibers, as denoted by their deeper-staining characteristics and by their displacement. This injury usually extended in width from 1 to 3 muscle fibers. In many of the injured muscle fibers there was definite indication of an attempt to regenerate, manifested by an increased number of muscle nuclei, as shown in figure 7 *A*. In most instances many of the regenerated nuclei appeared pyknotic, especially in the calcified fibers, yet many remained vesicular, with rounded ends.

The distribution of changes in the fibers varied considerably. In some fibers the first change was focal within the fiber, as indicated by a small round lesion in the fiber, while in others the whole fiber was involved. The early changes appeared to consist of some swelling and loss of striations of the fiber, which stained more deeply with eosin and sometimes more deeply with hematoxylin than the normal.

Later considerable fragmentation of the degenerated muscle fiber could be seen, probably followed in some instances by liquefaction. At this stage calcification began with continued swelling of the fiber. Sometimes the calcification appeared to take place at the periphery of the fiber first, but for the most part it appeared to be more or less diffuse. In cross sections there was often a halo effect around some of the swollen fibers, due to their shrinking during fixation. One case was encountered in which there was considerable degeneration of muscle without calcification, although there was calcification in the heart, aorta and splenic trabeculae of this animal.

The degeneration and calcification of the muscle of the diaphragm appeared to take place after the calcification of the pleural and peritoneal elastic elements of the diaphragm had started. The time relationship of the degeneration of other skeletal muscles of the body was not established.

*Liver.*—Blocks of liver tissue were saved from 19 animals, and the sections were all stained with eosin and alum-hematoxylin.

The most constant histologic alteration was a proliferation of fibroblasts in the periportal areas, indicative of the early stage of portal cirrhosis. This proliferation varied considerably in extent from animal to animal, one of the most marked examples being shown in figure 7 *B*. In most instances the reaction appeared to be widespread, indicating a diffusible injury, while in a few sections only 3 or 4 foci were found, indicative of a more focal condition. In a few sections there was some indication that the initial injury had taken place around the bile ducts and had later spread to include the portal vessels. In none of the cases had the injury been of sufficiently long standing to reach the stage of cicatrization. In three sections from different animals there was, in addition, some infiltration of these areas by lymphocytes and polymorphonuclears.

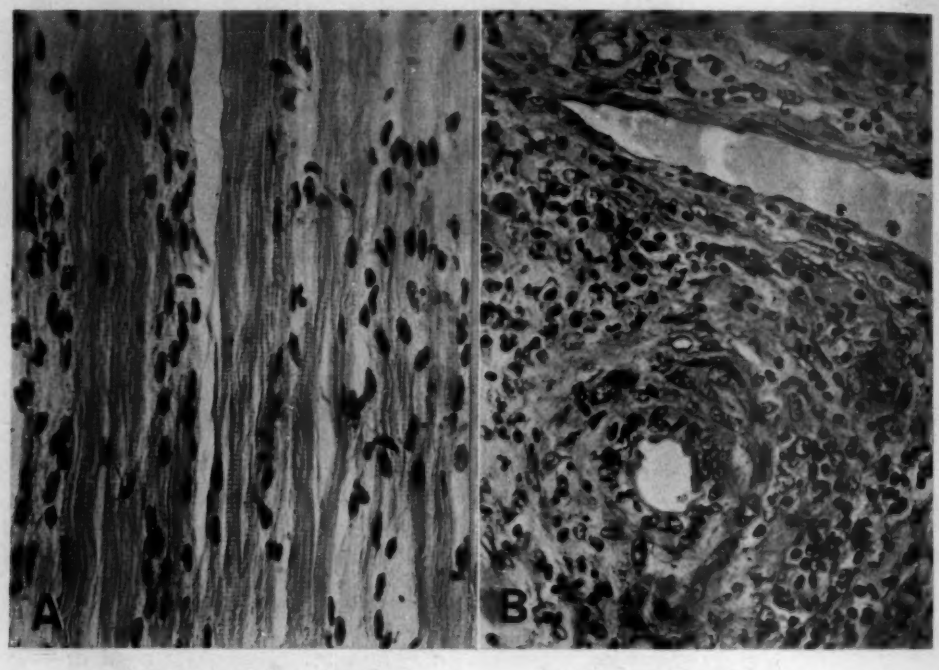


Fig. 7.—*A*, proliferation of the nuclei of skeletal muscle; eosin-alum-hematoxylin stain; × 350. *B*, proliferation of fibroblasts in the portal area of the liver; eosin-alum-hematoxylin stain; × 350.

The livers of 8 of the 19 animals showed atrophy of the parenchymatous cells surrounding the central veins. Four of the 8 showed varying degrees of chronic bronchopneumonia at autopsy, which may have contributed to this condition.

Three of the 19 showed fairly marked central necrosis of the parenchyma, which had not been of long standing. There was also some slight infiltration of these areas of injury by polymorphonuclears.

Seven showed microscopic pyemic foci, which might be expected in animals in such a poor state of health.

Nearly all the cases showed varying degrees of cloudy swelling and fatty degeneration.

*Kidney.*—Varying degrees of nephritis were present in most of the kidneys sectioned in this study. This material will be described in more detail at a later date. Therefore only a brief description of the changes will be given.

The most constant alteration was marked proliferation of fibroblasts and fibrosis of the interstitial tissue with marked atrophy and necrosis of the parenchyma, occurring in 12 of 21 cases from which renal tissue was saved. In 12 cases there were also varying degrees of focal infiltration of the interstitial spaces by lymphocytes, many occurring in the areas showing the fibroblastic reaction.

In 7 of the 21 cases marked tubular necrosis was observed, indicative of acute intoxication occurring a few days previous to death. In 6 the tubules showed deposits of calcium.

Other changes observed were some fibrosis of Bowman's capsule, proliferation of the glomerular capillary endothelium and deposits of débris in the subcapsular space. However, it was remarkable that there was comparatively slight injury of the glomerular structures in contrast with the marked injury of the interstitial and tubular tissue.

## COMMENT

Pathologic study of the tissues of calves that were raised on rations low in magnesium to the extent that the plasma magnesium was reduced to a low level showed as the principal change calcification of the yellow elastic tissue elements of the blood vascular system. The yellow elastic tissue elements of the surfaces of the diaphragm, of the spleen and of the trabeculae of the spleen were also involved.

The observations at autopsy indicated that the first changes probably took place in the endocardium and then extended through the larger arteries and veins. The smaller arteries, such as those found in the organs of the body, were not injured as a rule. Further study should be made on the extent of involvement of the blood vessels.

The histologic changes in the yellow elastic fibers may have been due directly to a deficiency of magnesium or to some change in metabolism produced by a deficiency of magnesium. The calves usually showed low plasma magnesium over a considerable period of time before the lesions developed.

Tufts and Greenberg [12] reported that when diets low in magnesium and high in calcium are fed to rats the abundance of calcium in the diet accentuates the effects of the deficiency in magnesium, and there was considerable retention of calcium in some of the tissues, especially the kidney. The calcium intake of the calves used in these experiments was not high but varied from normal to low. However, this change in calcium and magnesium metabolism may account for the deposition of calcium salts in the various tissues, as noted in this report.

It was difficult to establish definitely in some organs any evidence of histologic alteration in the yellow elastic fibers previous to calcifica-

12. Tufts, E. V., and Greenberg, D. M.: Proc. Soc. Exper. Biol. & Med. **34**:242, 1936.

tion. In the aorta it was possible to observe some fragmentation and degeneration of the yellow elastic fibers. The fact that the calcified fibers did not alter in staining reaction when Verhoeff's and Weigert's stains were used indicates that the injury to the fibers was not great even after calcification had taken place. Possibly the fiber had acquired some peculiar affinity for calcium salts and took up these salts from the flow of fluids which was nourishing it. There is the possibility, however, that these two stains have the same affinity for calcium salts as the healthy fiber, so that there was no differentiation between injured and uninjured fibers.

In some cases there was considerable fibroblastic reaction in the endocardium and in the intima of larger arteries. The reaction took place around the area of calcification, indicating a chronic type of irritation. No reaction was seen, however, in the media or adventitial coats of the aorta and larger arteries, nor was it noted in any other of the sites of calcification of the yellow elastic fibers.

The calcification of the myocardium and of the skeletal muscle was possibly secondary to the calcification of the vessels supplying blood to these tissues.

The degeneration and calcification found in the Purkinje fibers in some of the calves were probably a direct result of the deficiency of magnesium. Cardiographic studies are now in process to determine whether these changes are responsible for any alteration in cardiac rhythm.

Nephritis was not present in all the cases studied, but the type of injury noted, the fibroblastic reaction and fibrosis, indicate some type of chronic irritation, while the shape of the lesions indicates a vascular disturbance. The rather severe tubular injury noted in some cases indicates some type of rather acute intoxication occurring shortly before death.

The development of a fibroblastic reaction around the portal vessels of the liver indicates some type of chronic injury of the same order as that causing the fibroblastic reaction in the kidney. This condition in the liver was indicative of the first stage of portal cirrhosis.

The histologic changes found in calves fed diets low in magnesium bear some similarities to those reported observed in rats by other investigators. The pathologic observations reported by other investigators are not consistent. However, Greenberg and his associates [3] reported tubular necrosis in the kidney with fibrosis, a change which is noted in this report. Cramer [5] reported tubular degeneration and Brookfield [6] necrosis of the tubules. Schrader and his co-workers [4] reported perivascular accumulations of small round cells in the liver.

After studying the lesions associated with diets low in magnesium, in calves, one cannot help speculating as to the possible relationship of deficiency of magnesium to arteriosclerosis in man. After perusal of reviews such as those published by Duff,[13] Rosenthal[14] and Cowdry,[15] one must conclude that the exact cause of human arteriosclerosis is not known. At the outset we recognized some great differences in the two pathologic conditions, the one produced in young calves and the other found in human beings. On the other hand, there are some definite similarities.

In connection with the possible explanations of dissimilarities, it must be kept in mind that the work reported here was carried out on very young animals, of a species different from man. Species difference in reaction to the same deficiency is not an uncommon observation. Furthermore, in these experiments with calves the conditions produced were comparatively acute, whereas arteriosclerosis in man develops over a considerable period of time.

Although the rations fed the calves observed by Duncan, Huffman and Robinson[7] and by Huffman and Duncan[8] contained milk fat, calves showed the same lesions when fed on skim milk supplemented with starch, yeast and cod liver oil, so that a high fat ration was not a factor in producing the pathologic changes noted in this report.

The magnesium requirements for various animals have not been widely studied. Huffman and Duncan[8] showed that from 8 to 10 mg. of magnesium per pound of body weight as obtained from natural foods is required to maintain normal levels of plasma magnesium. Daniels and Everson[16] concluded, following balance trials on children, that the diet should contain not less than 13 mg. per kilogram of body weight. Coons and his co-workers[17] concluded from their own studies of metabolism on pregnant women and from data supplied by other workers that a safe range of magnesium intake is that from 0.35 to 0.45 Gm. daily, which would mean an intake of approximately from 6 to 8 mg. of magnesium per kilogram of body weight. An examination of the magnesium content of the common foods of the diet of man will reveal that the average intake is usually below these figures, especially if whole cereals and beans are not included in the diet. However, further data on human requirements must be collected before definite conclusions can be drawn.

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13. Duff, D. L.: Arch. Path. **20**:81 and 259, 1935.

14. Rosenthal, S. L.: Arch. Path. **18**:473 and 660, 1934.

15. Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

16. Daniels, A. L., and Everson, G. J.: J. Nutrition **11**:327, 1936.

17. Coons, C. M.; Schiefelbusch, A. T.; Marshall, G. B., and Coons, R. R.: Bulletin 223, Oklahoma Agricultural Experiment Station, 1935, p. 46.

We merely wish to point out and call to the attention of medical workers the possibilities of a relationship between magnesium deficiency and arteriosclerosis so that further observations may be made to prove or disprove the speculation set forth in this paper.

Aged cows are being placed on diets low in magnesium to study the effect of age on the alterations produced by the deficiency of magnesium, and work with rabbits in relation to the development of arteriosclerosis is also in progress.

## SUMMARY

Calves fed rations low in magnesium to the extent that the blood magnesium was reduced to a low level exhibited a definite pathologic picture. The principal pathologic alteration consisted of a deposition of calcium salts in the yellow elastic fibers of the endocardium, of the aorta, jugular vein and larger arteries, of the surfaces of the diaphragm and of the trabeculae and capsule of the spleen. Also notable were degeneration and calcification of Purkinje fibers. There were present also various degrees of hepatitis and nephritis. A possible relationship of diets low in magnesium to the arteriosclerosis of human beings is suggested.

Dr. C. F. Huffman, of the Department of Dairy Husbandry, provided us with the opportunity of collecting the tissues used in this study from his experimental animals.

Dr. W. T. S. Thorp, of the Department of Animal Pathology, took the photomicrographs used in this paper.

# FREQUENCY WITH WHICH SYPHILITIC LESIONS ARE ENCOUNTERED IN POSTMORTEM EXAMINATIONS

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MINNEAPOLIS

In view of the recent interest in the control of syphilis it seems important to determine the actual mortality from this disease. It is recognized that because of the stigma associated with syphilis vital statistics do not give an accurate record of deaths from this disease.

The frequency of syphilitic infection as determined by serologic tests is known to vary greatly with locality, race, age and sex. The disease is more frequent in urban than in rural communities and is much more common in Negroes than in white persons. In most adult populations, especially where white persons predominate, the infection is much more frequent in males than in females. In persons between 1 year and 18 years of age syphilitic infection is infrequent, and the incidence of the disease is low in persons past the age of 70 years.

A positive Wassermann reaction does not necessitate the presence of a syphilitic lesion that is demonstrable anatomically. In this department of pathology are records of a large number of cases in which positive serologic tests were not associated with anatomic changes. Obviously lethal syphilitic lesions are much less frequent than positive serologic tests. This study is concerned only with anatomically demonstrable syphilis; the actual incidence of the infection cannot be determined from the records studied.

## MATERIAL

The available material consisted of the records of 27,872 postmortem examinations made during the period from Jan. 1, 1910, to July 1, 1937. A much greater proportion of the material comes from the latter part of this period, since the annual number of postmortem examinations increased from 200 in 1910 to 2,500 in 1936.

The composition of the material as regards the age and sex of persons represented is shown in chart 1 and table 1. It will be noted in chart 1 that the curve for number of males in each age group corresponds rather closely to the general mortality curve which may be obtained by plotting the number of deaths in any large community by decades of life over a period of a few years. The curve for number of females in each age group, however, shows a dispropor-

From the Department of Pathology, University of Minnesota.
This study was aided by the Works Progress Administration.

tionately small number of women past the age of 30 years. As regards age and sex therefore the sample is uniform for all the groups except that of women over 30 years of age.

The sources of the material were: the University Hospitals, the Minneapolis General Hospital, the City and County Hospital of St. Paul (Ancker Hospital), one private hospital in St. Paul, all the private hospitals of Minneapolis, the private practice of all Minneapolis physicians who wish to utilize a free service, and the coroner's service of Hennepin County.

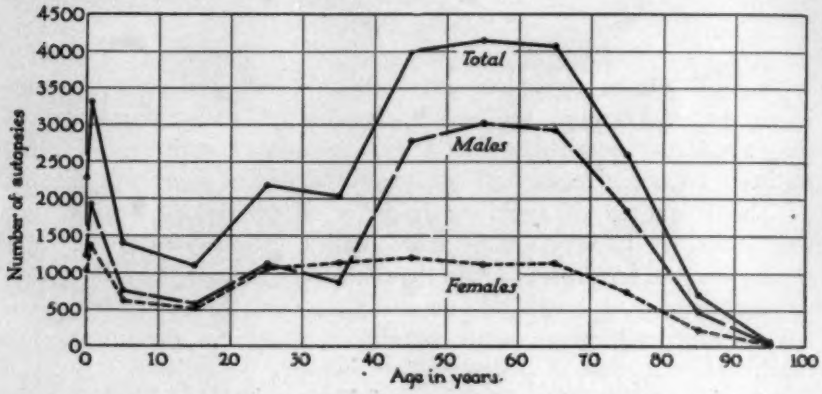


Chart 1.—Numbers of males and females in total number of persons examined post mortem at different decades of life.

TABLE 1.—*Distribution of Syphilitic Lesions with Reference to Age and Sex*

| Age Group, Yr. | Males | Syphilitic Males | | Females | Syphilitic Females | | Males and Females | Syphilitic Persons (Males and Females) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Per Cent | | Number | Per Cent | | Number | Per Cent |
| Stillborn*..... | 1,230 | 44 | 3.57 | 1,044 | 33 | 3.16 | 2,274 | 77 | 3.38 |
| 0 - 1*..... | 1,934 | 57 | 2.95 | 1,371 | 30 | 2.19 | 3,305 | 87 | 2.63 |
| 1 - 10*..... | 779 | 1 | 0.13 | 626 | 3 | 0.48 | 1,405 | 4 | 0.28 |
| 10 - 20..... | 572 | 3 | 0.52 | 531 | 1 | 0.2 | 1,103 | 4 | 0.36 |
| 20 - 30..... | 1,112 | 6 | 0.54 | 1,074 | 9 | 0.8 | 2,186 | 15 | 0.68 |
| 30 - 40..... | 887 | 72 | 8.12 | 1,145 | 26 | 2.27 | 2,032 | 98 | 4.82 |
| 40 - 50..... | 2,798 | 165 | 5.89 | 1,202 | 34 | 2.83 | 4,000 | 199 | 4.97 |
| 50 - 60..... | 3,026 | 138 | 4.56 | 1,125 | 34 | 3.02 | 4,151 | 172 | 4.14 |
| 60 - 70..... | 2,941 | 84 | 2.85 | 1,135 | 16 | 1.41 | 4,076 | 100 | 2.45 |
| 70 - 80..... | 1,843 | 11 | 0.59 | 747 | 2 | 0.27 | 2,590 | 13 | 0.50 |
| 80 - 90..... | 472 | 4 | 0.84 | 228 | 0 | 0.0 | 700 | 4 | 0.57 |
| 90 -100..... | 24 | 0 | 0.0 | 26 | 0 | 0.0 | 50 | 0 | 0.0 |
| Totals..... | 17,618 | 585 | 3.32 | 10,254 | 188 | 1.83 | 27,872 | 773 | 2.77 |

* Note that the first decade is subdivided into three groups: stillborn infants, infants under 1 year of age and children from 1 to 10 years old.

Since the first three hospitals listed admit few pay patients there is an undue proportion of persons in the lower economic levels, which probably gives an incidence of syphilis somewhat higher than would be obtained by a uniform sample of the community. It is to be remembered also that the great majority of the persons were urban residents. There were few Negroes in the group, not over 1 per cent.

The number of postmortem examinations made on residents of Minneapolis during the past five years equals about 16 per cent of the total number of deaths in the city during the same period. There is evidently some selection of individual diseases, since it is easier to obtain permission for a postmortem examination when the diagnosis is obscure than when it is well established clinically. As regards types of syphilis, the only known factor which would affect their proportionate representation in the postmortem observations is the fact that many residents of Minneapolis who have dementia paralytica die in state institutions outside the city and such persons would therefore be excluded from this study. The proportionate representation of dementia paralytica is consequently too low, but I know of no other factor which would influence the proportionate representation of the various types of syphilis.

## DIAGNOSIS

In the cases concerned in this study, congenital syphilis was diagnosed by its well known features. In a small percentage of the cases spirochetes were demonstrated. Splenomegaly with syphilitic osteochondritis was considered sufficient to establish the diagnosis. Splenomegaly with a positive serologic reaction in the mother or in the infant was accepted. In absence of both splenomegaly and syphilitic osteochondritis syphilis was not diagnosed unless spirochetes were demonstrated, even though the mother had a positive Wassermann reaction—there were few cases of this type. Care was taken to exclude erythroblastosis foetalis, which resembles congenital syphilis in that there is marked enlargement of the spleen.

Syphilitic aortitis was diagnosed only when the macroscopic changes were definite. In about 80 per cent of this group clinical symptoms were present. Probably some early cases of syphilitic aortitis were excluded since the diagnosis of an early case is often uncertain.

Neurosyphilis in practically all cases was clearly established by the clinical symptoms, and in nearly all of the cases the diagnosis was confirmed by microscopic examination of the nervous system.

## SYPHILIS AS A CAUSE OF DEATH

Syphilis was the major cause of death in all of the 170 cases of congenital syphilis and in all of the 179 cases of neurosyphilis, but in 61 of the 387 cases of syphilitic aortitis, in 14 of the 20 cases of hepar lobatum and in 6 of the miscellaneous group the syphilis was apparently not a contributory cause of death. Therefore, if these 81 cases are removed from table 1, the records show about 2.5 per cent of all deaths due to syphilis.

## DISTRIBUTION OF DEATHS FROM SYPHILIS IN DIFFERENT DECADES OF LIFE

The age distribution of lethal syphilis is shown in table 1. It appears that 3.38 per cent of the stillborn infants were syphilitic and that 2.63 per cent of deaths under 1 year of age were due to syphilis. In the latter group 76 of the 87 infants were under 3 months of age. It is surprising to note that after the age of 1 year congenital syphilis is a

negligible factor in the death rate. This may mean either that congenital syphilis is usually fatal or that infants that survive as long as one year ultimately recover.

There were comparatively few deaths from syphilis during the second and third decades of life. In the second decade there were 4 deaths from congenital syphilis (table 2). In the third decade, in which most of the infections are believed to be acquired, there were 10 deaths from syphilitic aortitis, 1 from meningovascular syphilis and 1 from arsenical therapy. In 3 instances syphilis was at most only a contributory cause of death.

TABLE 2.—*Distribution of Various Types of Syphilitic Lesions in Relation to Age at Time of Death*

| | | | Persons in Whom Given Form of Syphilis Was Observed | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Neurosyphilis | | | | | | |
| Age Group, Yr. | Autopsies | Persons in Whom Syphilitic Lesions Were Noted | Tabes and the Tabetic Form of Dementia Paralytica | Dementia Paralytica | Meningovascular Syphilis | Congenital Syphilis | Syphilitic Aortitis | Hepar Lobatum | Miscellaneous |
| Stillborn... | 2,274 | 77 | 0 | 0 | 0 | 77 | 0 | 0 | 0 |
| 0 - 1..... | 3,305 | 87 | 0 | 0 | 0 | 87 | 0 | 0 | 0 |
| 1 - 10..... | 1,405 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 10 - 20..... | 1,103 | 4 | 0 | 1 | 1 | 2 (4) | 0 | 0 | 0 |
| 20 - 30..... | 2,186 | 15 | 0 | 0 | 1 | 0 | 10 | 0 | 4 |
| 30 - 40..... | 2,032 | 98 | 12 | 6 | 17 | 0 | 59 | 4 | 6 |
| 40 - 50..... | 4,000 | 199 | 14 | 14 | 34 | 0 | 130 | 4 | 3 |
| 50 - 60..... | 4,151 | 172 | 23 | 9 | 13 | 0 | 114 | 10 | 3 |
| 60 - 70..... | 4,076 | 100 | 14 | 3 | 11 | 0 | 70 | 1 | 1 |
| 70 - 80..... | 2,590 | 13 | 2 | 0 | 1 | 0 | 9 | 1 | 0 |
| 80 - 90..... | 700 | 4 | 2 | 0 | 1 | 0 | 1 | 0 | 0 |
| 90 -100..... | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 27,872 | 773 | 67 | 33 | 79 | 170 | 387 | 20 | 17 |

179

The maximal death rate from acquired syphilis occurs in the fourth, fifth and sixth decades of life. The distribution of syphilis by age and sex is shown in table 1 and chart 2.

### DISTRIBUTION OF DEATHS FROM SYPHILIS IN MALES AND FEMALES

There is no significant difference in the incidence of lethal congenital syphilis related to sex, but acquired syphilis causes approximately twice as many deaths in males as in females (table 1 and chart 2).

### DISTRIBUTION OF DIFFERENT TYPES OF SYPHILITIC LESIONS IN POSTMORTEM MATERIAL

The distribution of the various forms of syphilitic disease according to decades of life is shown in table 2 and chart 3. There is some overlapping of neurosyphilis and syphilitic aortitis, but each case is listed only once. In 42 of the 179 cases of neurosyphilis a subclinical syphilitic

aortitis was present, and in 7 cases in which death was due to syphilitic aortitis neurosyphilis was present. There is no striking difference in the distribution of neurosyphilis or of syphilitic aortitis related to the decade of life at the time of death, and the data do not justify the conclusion that neurosyphilis is a later manifestation of syphilitic infection than is syphilitic aortitis.

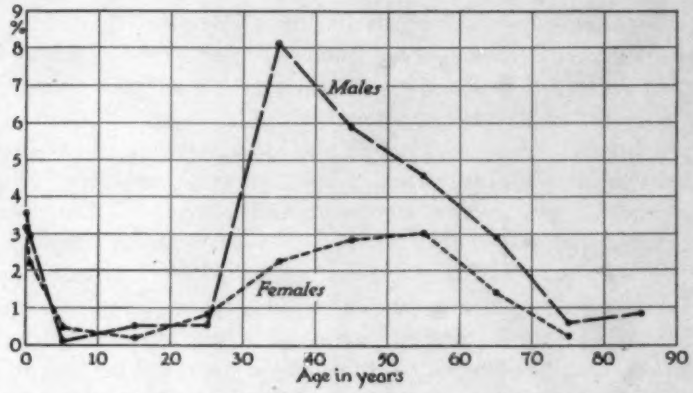


Chart 2.—Distribution of syphilis in males and females examined post mortem at different decades of life.

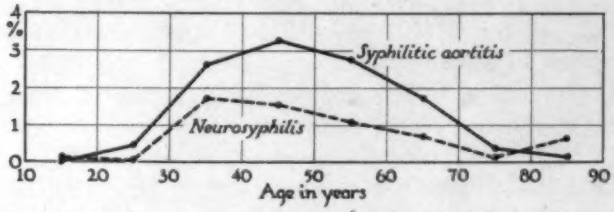


Chart 3.—Distribution of neurosyphilis and syphilitic aortitis in persons examined post mortem at different decades of life.

## SUMMARY

In a survey of 27,872 postmortem examinations it was found that definite syphilitic lesions were noted in 2.77 per cent, and in approximately 2.5 per cent syphilis was shown to be the major cause of death. About 3.38 per cent of stillborn infants were syphilitic, and congenital syphilis caused about 2.63 per cent of the deaths in infants under 1 year of age. Congenital syphilis was a rare cause of death in infants more than 1 year of age.

Syphilis was an infrequent cause of death in persons dying in the second and third decades of life. The maximal mortality from this cause occurred in persons in the fourth, fifth and sixth decades of life.

Acquired syphilis, in its lethal forms at least, was about twice as frequent in males as in females.

The major forms of lethal syphilis were syphilitic aortitis and neurosyphilis, and the age distribution of these two forms was about the same.

# A PIGMENTED ADENOMA OF THE ADRENAL

MARGARET R. BAKER, M.D.
AKRON, OHIO

It is difficult to identify positively the various pigments that are found in the body in health and in disease, since the majority are some shade of brown in sections prepared with routine stains and often react similarly with special stains. The only brown pigments commonly recognized as occurring in tumors are melanin and hemosiderin. The latter is easily identified with stains for iron, but tumors with noniron-containing pigment are usually diagnosed as melanoma without any further study of the pigment. This is reasonable as regards pigmented tumors of the eye and skin and even of those arising from the nose, mouth, vagina, rectum, meninges and brain, since melanin-bearing cells have been demonstrated in these regions. But when the liver, kidneys, adrenals, intestines or ovaries are cited as the origin of melanotic tumors, as all have been, there is need for skepticism unless the pigment can be identified unquestionably as melanin.

In the literature are several reports of the adrenal glands as the site of tumors considered to have been malignant melanoma. Millar [1] collected 4 such reports published between 1911 and 1915. In 2 of the cases the tumor resembled the usual melanosarcoma of the skin, and in 2 the growth appeared to be composed of ganglion cells containing melanin. Smith [2] described a bilateral melanotic growth of the adrenals with secondary growths in the intestines, spleen and mesenteric lymph nodes. McComb and Smith [3] reported a melanotic sarcoma involving both adrenals, with secondary tumors in the bladder and in the subcutaneous tissues of the neck.

Recently a benign pigmented adenoma of the adrenal was discovered at autopsy.

## REPORT OF CASE

A 48 year old white housewife was admitted to the Western Pennsylvania Hospital, to the service of Dr. J. N. Stanton, Feb. 4, 1937. Her only complaint was of severe pain in the epigastrium. She had been well until about a year before, when she began to have a sensation of aching in the upper midregion of the abdomen, coming on at irregular intervals and lasting for from three to

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From the Institute of Pathology of the Western Pennsylvania Hospital, Pittsburgh.

1. Millar, W. G.: J. Path. & Bact. **35**:351, 1932.
2. Smith, R. M.: M. J. Australia **1**:683, 1927.
3. McComb, R. A., and Smith, D. B.: J. Urol. **30**:49, 1933.

seven days at a time. This aching gradually increased in severity and had a tendency to radiate to both sides beneath the costal margin. It was not relieved by either food or sodium bicarbonate.

Her physician told her that she had disease of the gallbladder and put her on a fat-free diet. She never had clay-colored stools, jaundice, right-sided colic, nausea or vomiting. She lost 8 pounds (3.6 Kg.) during this year, a loss which she attributed to the fat-free diet. In spite of the diet and various types of medication the epigastric pain steadily increased in severity until at the time of admission it was intense and practically constant.

She had had 10 children, 3 of whom died in infancy. Recently the menstrual periods had been irregular.

On admission the temperature was 98.8 F., the pulse rate 104 and the respiratory rate 20. The head, neck and chest were normal. There was a slight fulness in the right upper quadrant of the abdomen, and the edge of the liver could be felt 3 fingerbreadths below the costal margin. A hard mass was palpable in the region of the gallbladder. Neither the liver nor the mass was tender. The red blood cells numbered, 4,620,000, and the hemoglobin content was 90 per cent. The white cell count was 7,800, with a normal differential picture. The urine showed a trace of albumin and many leukocytes. The Kahn test was negative.

A roentgen study of the gastrointestinal tract revealed that the abdominal mass had pushed the stomach to the left and the transverse colon downward but that there was no connection between the mass and the alimentary canal. The right side of the diaphragm was elevated and not as freely movable as the left.

February 10 an exploratory laparotomy revealed a large fixed retroperitoneal cystic tumor which pushed the liver anteriorly and to the left and the right kidney downward. An attempt was made to take a piece of tissue from it for biopsy, but because of excessive bleeding this was abandoned. About 300 cc. of dark red liquid blood was withdrawn by needle from the center of the mass. This blood was centrifuged and the sediment examined for tumor cells, but none were found.

Following the operation the patient lived for seventeen days, gradually losing strength. The hemoglobin content immediately after operation was 70 per cent. The patient had daily a slight, irregular elevation of temperature and a rapid pulse, varying from 120 to 140. The right leg became swollen to about twice the size of the left. February 27 she died rather suddenly after a rapid rise of temperature to 105 F. in the space of a few hours.

*Postmortem Examination.*—An autopsy was made nine hours after death. Numerous fibrous adhesions were found about the operative site, involving the liver, colon and a retroperitoneal mass, reddish blue and cystic, which occupied most of the right upper half of the abdomen and extended beneath the liver. The latter in consequence was much flattened in its anterior-posterior diameter. This huge cyst measured 12 by 15 by 34 cm. and was bounded above by the liver, below by the kidney and medially by the inferior vena cava. The right kidney was pushed downward and its upper pole flattened. As there was no invasion of any of the adjacent organs the cyst was easily dissected free from these structures. Its external surface was bluish red, smooth, rounded and faintly lobulated. On section the cyst wall varied from 4 mm. to 2 cm. in thickness and was an exceedingly dark homogeneous red. The inner surface was rather ragged, though occasionally it seemed to be covered by a thin smooth grayish membrane. The lumen contained dark red fluid blood and some soft red blood clots.

The inferior vena cava was bound rather tightly to the cyst and received a large vein from it. Distal to this branch it was occluded by an adherent friable yellow thrombus 4 cm. long. There were no thrombi in the iliac veins. The other organs were essentially normal except for early bronchopneumonia.

*Microscopic Examination.*—The tissue of the cyst wall was well preserved and, while hemorrhages were present, they were not nearly as extensive as suggested by the gross appearance. Rounded groups of epithelial cells were separated from each other by a network of thin-walled, dilated blood vessels of varying diameter with an accompanying delicate connective tissue stroma. A few coarse fibrous trabeculae were seen, and there was a well marked fibrous tissue capsule. The epithelial cells were irregularly polygonal, of medium size, with fairly abundant granular pink cytoplasm and small round or oval dark nuclei, each with a prominent nuclear membrane and rather finely divided chromatin. The epithelial islands were sometimes compact, but usually the cells were quite loosely arranged in small ragged groups, separated by irregular empty spaces (fig. 1*A* and *B*). The morphologic character of the cells was uniform, and there were no mitotic figures.

Medium-sized dark brown granules were found in the majority of the cells, sometimes densely packed together but more frequently widely enough spaced that some of the pink cytoplasm could be seen. These granules appeared almost black under low power but varied from yellowish brown to dark brown under high power, the majority being quite dark. They showed little variation in size. They did not give a positive reaction for iron by the berlin blue reaction. In the stroma there were a few phagocytic cells containing iron pigment. However, this pigment was present in larger and extremely variable-sized globules of a much lighter brown. The diagnosis was a pigmented adenoma of the adrenal, probably a melanoma.

In order to determine more accurately the origin of the tumor and the nature of the pigment contained in it a number of special staining methods were employed. Frozen sections stained for fat with scarlet red showed only an occasional cell which contained fat; this was present in the form of small, rather pale, pink globules. At the time of the autopsy none of the tissue was fixed in potassium bichromate. Several days later some tissue was fixed in a 5 per cent aqueous solution of this salt. A negative chromaffin reaction was obtained but could not be relied on because of the long lapse of time between death and fixation of the tissue. Since the sinusoids of the medulla are lined with elastic fibers, while those of the cortex are not, sections were stained with Weigert's elastic tissue stain, and it was found that the thin-walled blood vessels of the tumor contained no elastic fibers.

In studying the brown pigment in the tumor the following stains were used: the berlin blue stain for iron, with a counterstain of 0.5 per cent basic fuchsin; 1 per cent aqueous toluidine blue; Foot's ammoniacal silver stain for paraffin sections; 5 per cent aqueous silver nitrate heated to 37 C. for several hours. Unstained sections were bleached with hydrogen peroxide over a period of several days, and unstained sections were treated with acetone and chloroform to determine whether the pigment was fat soluble. These stains were also tested on sections of Negro skin, nevi, malignant melanomas, pigmented basal cell carcinomas, ganglion cells of the substantia nigra and of sympathetic ganglions, melanosis coli (1 case), old hemorrhagic ovarian cysts and heavily pigmented adrenals, hearts and livers from middle aged and elderly people (table).

Hemosiderin was found to be the most easily recognized of the brown pigments because, in addition to giving a positive reaction for iron, it was the only brown pigment tested which did not stain green with toluidine blue and which did not bleach with hydrogen peroxide. Hemofuscin had one outstanding reaction, namely, its deep red stain with basic fuchsin.

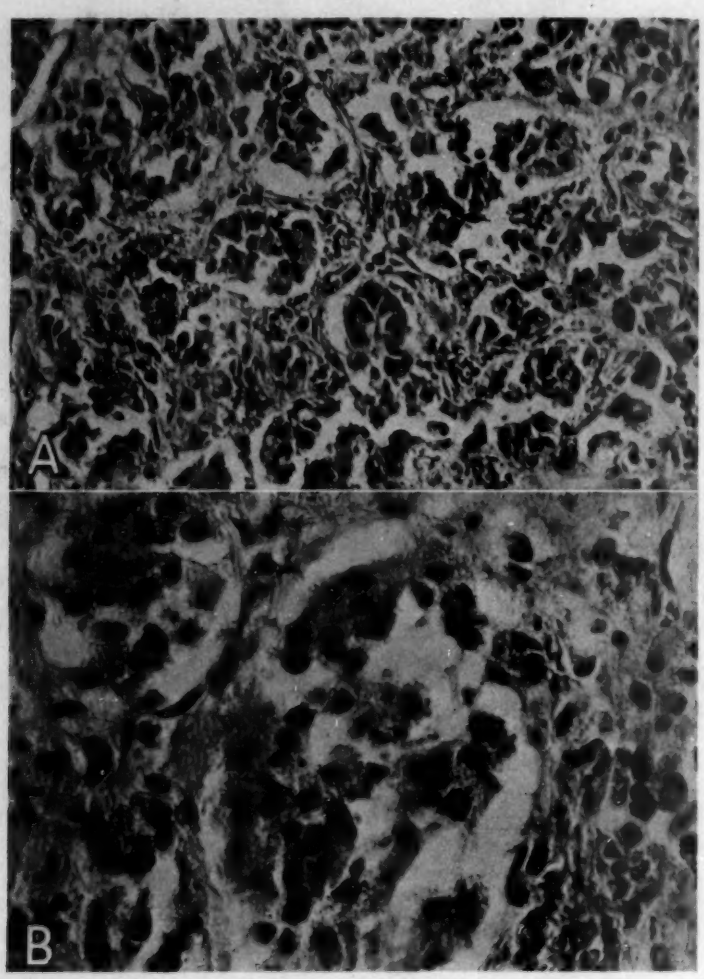


Fig. 1.—*A*, low power photomicrograph showing the general architecture of an adrenal tumor; hematoxylin and eosin; × 225. *B*, high power photomicrograph to show the cellular appearance of the tumor; hematoxylin and eosin; × 500.

Melanosis coli and the pigment found in brown atrophy of the heart and with increasing age in the liver cells and in the zona reticularis of the adrenal was chiefly distinguished from melanin by its lack of staining with aqueous silver nitrate even when kept for twenty-four hours at 37 C.

The rest of the brown pigments tested, including that of the adrenal adenoma presented here, were apparently all melanins and gave similar staining reactions, the most important of which was a blackening of the pigment with 5 per cent aqueous silver nitrate. The identification of melanin by means of aqueous silver nitrate is advocated by Millar[1] and Becker.[4] Becker used 2 per cent silver nitrate at room temperature in the light for two hours. His results were somewhat erratic, many malignant melanomas showing no darkening of their pigment, although he usually obtained positive results with cutaneous melanin and nevi. Millar used 5 per cent silver nitrate, but his paper gives no details as to method or results.

With 5 per cent silver nitrate on pieces of skin and melanotic tumors, it was found that variable results were obtained at room temperature, due to day by day fluctuations. Much more satisfactory stains were obtained by placing the slides in the incubator at 37 C. At this temperature there was definite darkening of

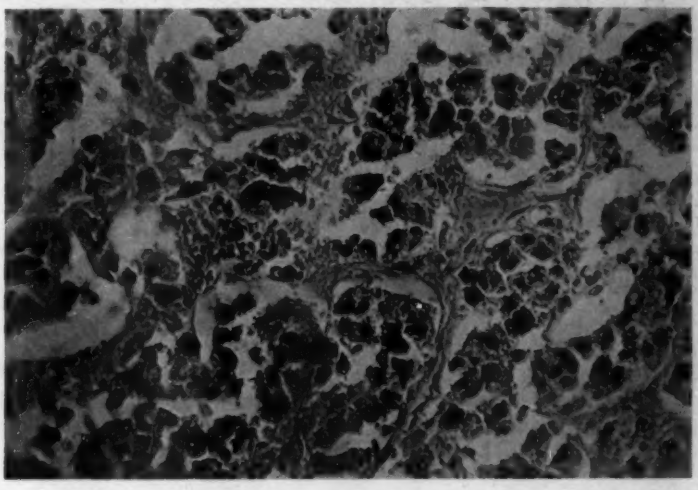

Fig. 2.—Adrenal tumor stained with 5 per cent aqueous silver nitrate to accentuate pigment granules; × 260.

melanin in an hour or less, and the reaction usually reached its height in two hours. The pigment appeared black under low power and very dark brown under high power. This reaction was always positive in cutaneous epithelium, nevi, malignant melanomas and pigmented basal cell carcinomas. In tumors with scanty pigmentation it rendered the pigment conspicuous and thus was a valuable aid to diagnosis. The melanin in the skin usually darkened a little more rapidly than the melanin in tumors, particularly that in malignant melanoma, but the end result was about the same. A somewhat less intense darkening occurred in the ganglion cells of the substantia nigra. The pigment in the ganglion cells of the sympathetic ganglions in the adrenal capsule was definitely but rather slowly blackened, the maximal reaction occurring in about six hours.

The demonstration of melanin by ammoniacal silver stains, such as the Bielschowsky and its modifications, is advocated by many authors, including

4. Becker, S. W.: Arch. Dermat. & Syph. **16**:259, 1927.

Masson,[5] Mallory and Wright,[6] Jacobsen and Klinck,[7] Foot[8] and Smith.[9] These stains were found to be absolutely nonspecific since they stained every brown pigment tested, including hemosiderin, a deep black.

The pigment in the adrenal adenoma was found to give all the staining reactions of other known melanins. It agreed most closely with cutaneous melanin in its rapid blackening with aqueous silver nitrate (fig. 2) and its fairly rapid bleaching with hydrogen peroxide. It was found to be more rapidly soluble in potassium hydroxide than any other skin or tumor melanin tested.

*Staining Characteristics of Brown Pigments*

| Pigment (Designated by Location) | Solubility in Fat | Staining with Fat Stains | Staining with 1% Aqueous Toluidine Blue | Staining with 0.5% Basic Fuchsin | Iron Content | Bleaching with Hydrogen Peroxide | Staining with Ammoniacal Silver Stains | Staining with 5% Aqueous Silver Nitrate |
|---|---|---|---|---|---|---|---|---|
| Group A | | | | | | | | |
| Pigmented adrenal tumor | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black |
| Negro skin (melanin) | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black |
| Nevi..... | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black |
| Pigmented basal cell carcinoma | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black |
| Malignant melanoma | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black |
| Group B | | | | | | | | |
| Ganglion cells of substantia nigra | 0 | 0 | Green | 0 | 0 | + | Black | Very dark brown |
| Sympathetic ganglion cells | 0 | 0 | Green | 0 | 0 | + | Black | Brown-black (slow reaction) |
| Group C | | | | | | | | |
| Melanosis coli.... | 0 | 0 | Green | 0 | 0 | + | Black | 0 |
| Pigmented livers, hearts and adrenals | 0 | 0 | Green | 0 | 0 | + | Black | 0 |
| Group D | | | | | | | | |
| Hemofuscin..... | 0 | 0 | Green | Red | 0 | + | Black | 0 |
| Group E | | | | | | | | |
| Hemosiderin..... | 0 | 0 | 0 | 0 | + | 0 | Black | 0 |

## COMMENT

The morphologic character of this adrenal tumor was entirely different from that of melanoma in most cases. Melanin, however, is not characterized as the property of any single type of cell, being produced by cells of both connective and epithelial tissues of diverse locations. Chemical examination, moreover, has tended to show that melanins are

5. Masson, P.: Bull. Acad. de méd., Paris **109**:22, 1933.

6. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924.

7. Jacobsen, V. C., and Klinck, G. H.: Arch. Path. **17**:141, 1934.

8. Foot, N. C.: Am. J. Path. **8**:309, 1932.

9. Smith, L. W.: Arch. Path. **1**:365, 1926.

not all one specific chemical substance but a group of closely allied compounds. A few melanins contain a small amount of iron (not stainable). Many contain sulfur. According to Wells,[10] this may be as high as 12 per cent in the squid and 10 per cent in the melanotic sarcoma in some instances, and from 2 to 4 per cent in hair; it is absent in melanin from the choroid. However, Bloch [11] said that the iron and sulfur are probably impurities.

A number of colorless organic cyclic compounds, such as phenol, pyrogallol and tyrosine, may be changed to melanins by the oxidizing ferments produced by various plants. The oxidizing ferment ordinarily present in melanoblasts in man apparently acts only on 3, 4-dioxyphenylalanine (dopa). However, Wells reported that Neuberg had produced pigment from epinephrine and betaoxyphenylethylamine with extracts of a melanosarcoma of the adrenal, that Alsberg had oxidized pyrocatechin (biscatecholdisulfonate) to a pigment with an enzyme in a melanosarcoma of the liver and that Jager had oxidized epinephrine to a pigment with extracts of a melanosarcoma from a horse. If the adrenal tumor concerned in this report was of medullary origin—of which there is no good evidence—it is conceivable that its melanin might have been produced by the oxidation of epinephrine.

The pigment in this adrenal adenoma was exceedingly dark, much darker than that found in the majority of melanomas. A somewhat similar case was reported by Smith.[9] He observed a primary melanotic sarcoma of the liver which contained blackish brown pigment. It was, however, not absolutely identified as melanin.

In substantiating the diagnosis of a melanotic tumor of unusual character and location most authors consider it sufficient to state that the pigment in question is iron negative, bleaches with hydrogen peroxide or ferric chloride and stains black with ammoniacal silver stains. A few writers have noted that melanin stains green with toluidine blue and is darkened by aqueous silver nitrate. None of these stains except the iron reaction and the blackening of the pigment by aqueous silver nitrate gives any valuable information. While it cannot be proved that no substance except melanin stains black with aqueous silver nitrate, there is no good reason at present to doubt the specificity of this stain. It is a helpful adjunct to Bloch's dopa stain. Melanoblasts may be demonstrated by the dopa reaction, but this requires fresh tissue and presupposes a suspicion that the tumor contains melanin. In the case reported here the hemorrhagic appearance of the tissue masked its black color, and by the time the tumor was recognized as a possible melanoma it was too late to try for a dopa reaction.

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10. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

11. Bloch, B.: Am. J. M. Sc. **177**:609, 1929.

The nature of the "wear and tear" pigments in the liver, heart and adrenals has never been definitely settled. Wells refers to them as lipochromes or lipofuscins. In the present studies, however, these pigments were not fat soluble and did not stain with fat stains, although they were practically always present in cells which contained fine globules of fat intimately mixed with the pigment granules. Mallory and Wright stated that these pigments are melanins on the ground that they contain no iron or fat, bleach with sunlight and oxidizing reagents, are soluble in alkalis and are stained black by ammoniacal silver stains. However, in the course of this investigation these pigments were found to differ radically from melanin in not staining with aqueous silver nitrate.

Before the silver nitrate stains were done and their specificity appreciated, it was thought that the tumor probably arose from the zona reticularis of the cortex. The brown pigment in this zone is sometimes dark and abundant. The small amount of lipoid in the tumor cells fitted in fairly well, since the cells of the zona reticularis contain less lipoid than the rest of the cortex, and occasional groups of cells are seen which contain practically none. The eosinophilic cytoplasm and small dark round nuclei of the tumor cells suggested adrenal cortex rather than medulla, and the lack of elastic tissue in the sinusoids of the tumor also seemed in favor of a cortical origin. However, the somewhat ragged borders of the cells, the fact that the production of melanin often seems related to epinephrine, and the inconclusiveness of the chromaffin reaction made it impossible to rule out a medullary origin.

A melanotic tumor in the adrenal gland may be explained in several ways, depending on the type of cell forming the tumor. If the tumor had its origin from ganglion cells either in the surrounding ganglions or in neurons in the medulla, the formation of melanin would present no difficulties. Melanin-bearing fibroblasts have been reported in the capsule of the adrenal of the guinea pig and occasionally in that of man by Smith.[9] Since the adenoma presented here obviously was not derived from ganglion cells or fibroblasts but from epithelial cells, it seems that the only alternative is to consider that the melanin was formed from epinephrine and that, therefore, the origin of the tumor was probably the adrenal medulla.

SUMMARY

An unusual adenoma of the adrenal gland contained a brown pigment which gave all the staining reactions of melanin.

The best differential stain for melanin was found to be 5 per cent aqueous silver nitrate used at 37 C.

# MAJOR DUODENAL PAPILLA

## VARIATIONS OF PATHOLOGIC INTEREST AND LESIONS OF THE MUCOSA

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The duodenal papilla was recently investigated anatomically and histologically in 100 cases. In this preliminary study special attention was given to the mucosa, and numerous anatomic and histologic variations were found which are of importance to those interested in the pathology of the major papilla. The more significant of these variations were: (1) the manner in which the bile duct and pancreatic duct entered the duodenum, (2) the character of the epithelial transitions within the major papilla and (3) the presence of adenomatoid hyperplasia at the orifice of the papilla.

The manner in which the ducts entered the duodenum in 55 cases is shown diagrammatically in figure 1. In 19 cases the two ducts opened separately through a single papilla. In 2 cases there were two openings at widely separated points on the same papilla. In 1 case the pancreatic duct formed a sinus about the bile duct 3 mm. from the apex (fig. 1*d*). In 33 cases the bile duct and pancreatic duct joined within the papilla at variable distances from the orifice. The distances from the orifice at which the junctions occurred are shown in table 1.

It is apparent from these observations that an ampulla in the sense of being a dilated reservoir into which the bile duct and pancreatic duct empty is an infrequent finding. Thus, there were only 6 cases in which the junction of the ducts occurred 5 mm. or more from the orifice. The findings of other workers are summarized in table 2, which shows that in 43 per cent of the 1,001 specimens examined there was no ampulla. It should also be pointed out that in many of the 57 per cent in which an ampulla was present the junction of ducts did not occur beyond 1 or 2 mm. from the apex of the papilla. The significance of these facts in regard to certain theories of the causation of pancreatitis will be pointed out later in this paper.

In all the specimens examined there was marked narrowing of the ducts as they pierced the duodenal wall to enter the papilla. The extent

of this narrowing as observed in 55 cases is shown in table 3. This abrupt reduction of caliber was owing largely to the distribution of the sphincteric and duodenal musculature at this point, but it was attrib-

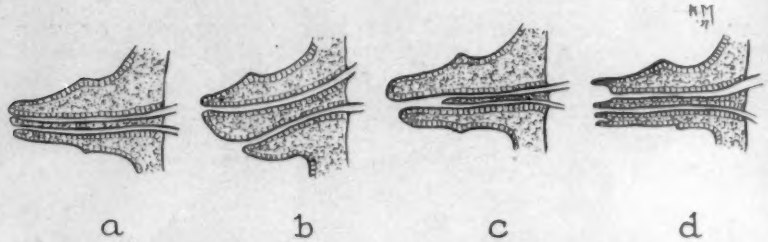


Fig. 1.—The manner in which the ducts opened into the duodenum: *a*, 19 cases; *b*, 2 cases; *c*, 33 cases; *d*, 1 case.

TABLE 1.—*Cases in Which Ducts Joined (Fig. 1c)*

| Distance from Outlet, Mm. | Cases |
|---|---|
| 2 | 11 |
| 3 | 3 |
| 4 | 13 |
| 5 | 5 |
| 7 | 1 |

TABLE 2.—*Summary of One Thousand and One Cases*

| Investigator | Cases in Which Ducts Joined | Cases in Which Ducts Did Not Join |
|---|---|---|
| Schirmer, A. M., 1898, cited by Mann and Giordano | 25 | 22 |
| Letulle, M., and Nattan-Larrier: Bull. Soc. anat. de Paris **73**:491, 1898 | 9 | 12 |
| Opie, E. L.: Bull. Johns Hopkins Hosp. **14**: 229, 1903 | 89 | 11 |
| Ruge, E.: Arch. f. klin. Chir. **87**: 47, 1908 | 32 | 11 |
| Baldwin, W. M.: Anat. Rec. **5**: 197, 1911 | 70 | 20 |
| Belou, P., 1915, cited by Mann and Giordano * | 15 | 27 |
| Mann, F. C., and Giordano, A. S.: Arch. Surg. **6**: 1, 1923 | 138 | 62 |
| Cameron, A. L., and Noble, J. F.: J. A. M. A. **82**: 1410, 1924 | 74 | 26 |
| Nagai, K., and Sawada, T.: Acta scholae med. univ. imp. in Kioto **8**: 91, 1925 † | 52 | 5 |
| Job, T. T., abstracted, Anat. Rec. **32**: 212, 1926 | 8 | 143 |
| Holzapfel, R.: Anat. Anz. **69**: 449, 1930 | 11 | 39 |
| Dardinski, V. J.: J. Anat. **69**: 469, 1935 | 47 | 53 |
| Total | 570 | 431 |
| Percentage | 57 | 43 |

* The original article was not obtainable, and it was necessary to derive the numbers from quantities expressed in percentages by authors who cited Belou.

† The pancreatic duct was obliterated in one case (total number of cases 58).

TABLE 3.—*Circumference of Ducts of Fifty-Five Adults*

| Point Measured in Relation to Duodenal Wall | Bile Duct, Mm. | | | Pancreatic Duct, Mm. | | |
|---|---|---|---|---|---|---|
| | Mean | Largest | Smallest | Mean | Largest | Smallest |
| Before entrance | 10.0 | 10.7 | 6 | 5.5 | 10.0 | 3 |
| At entrance | 6.3 | 9.0 | 5 | 4.1 | 8.0 | 2 |
| After entrance | 5.4 | 1.0 | 4 | 4.3 | 7.0 | 2 |

utable also, in some measure, to the increased thickness of the mucous membrane in the intrapapillary portion of the ducts. In some cases the narrowing was so pronounced that it was impossible to insert even a small probe without applying considerable force. In the opened duct the abrupt change in caliber was indicated in some cases by a definite ridge or step at the junction of the extraduodenal and intraduodenal portions of the ducts.

Examination of the opened papilla revealed also an abrupt transition in the character of the mucosa at the point where the ducts penetrated the duodenal wall. The extraduodenal portions of the ducts, except for the parietal sacculi of Beale in the common bile duct, were comparatively smooth and flat. The intrapapillary portions of both ducts, however, presented a rough, shaggy appearance, which on closer examination was seen to be due to numerous mucosal folds that coursed longitudinally and obliquely through the ducts. Many authors refer to these folds as valves, and their appearance is certainly suggestive of a valvular function. In the terminal portion of the ducts, for a distance of from 1 to 3 mm., the folds were not attached to the walls by a broad base but hung free as papillary processes. They were either long and slender or short and thick; some were branched, and many were club shaped. In 4 of the 55 specimens which were examined under the dissecting microscope, these free folds or papillary processes extended from 2 to 3 mm. beyond the orifice. In some cases the processes were so numerous that they appeared to block the outlet. When they were long enough to attract attention grossly, as these were, they were called polyps. They extended from the wall and also from the septum between the two ducts. In fresh specimens they appeared pink or red, congested and fleshy.

The epithelial transitions within the papilla were studied microscopically in 45 specimens. It was found that the transition from bile duct to pancreatic duct mucosa was practically imperceptible in those cases in which the ducts joined, and no definite point could be determined that indicated where the epithelium of the bile duct began and the epithelium of the pancreatic duct ended and vice versa (fig. 2 *A*). The transition from the mucosa of the duct or ampulla to duodenal mucosa was a more abrupt and interesting one, however (figs. 2 *A* and *B*). The duodenal mucosa in the region immediately surrounding the outlet was covered by a sheath of epithelium with small villi. Occasionally the villi were missing altogether. In contrast with this, the intrapapillary mucosa presented numbers of papillary processes much larger than the duodenal villi. The lining epithelium of the duodenum was markedly eosinophilic as contrasted with that of the ducts or ampulla.

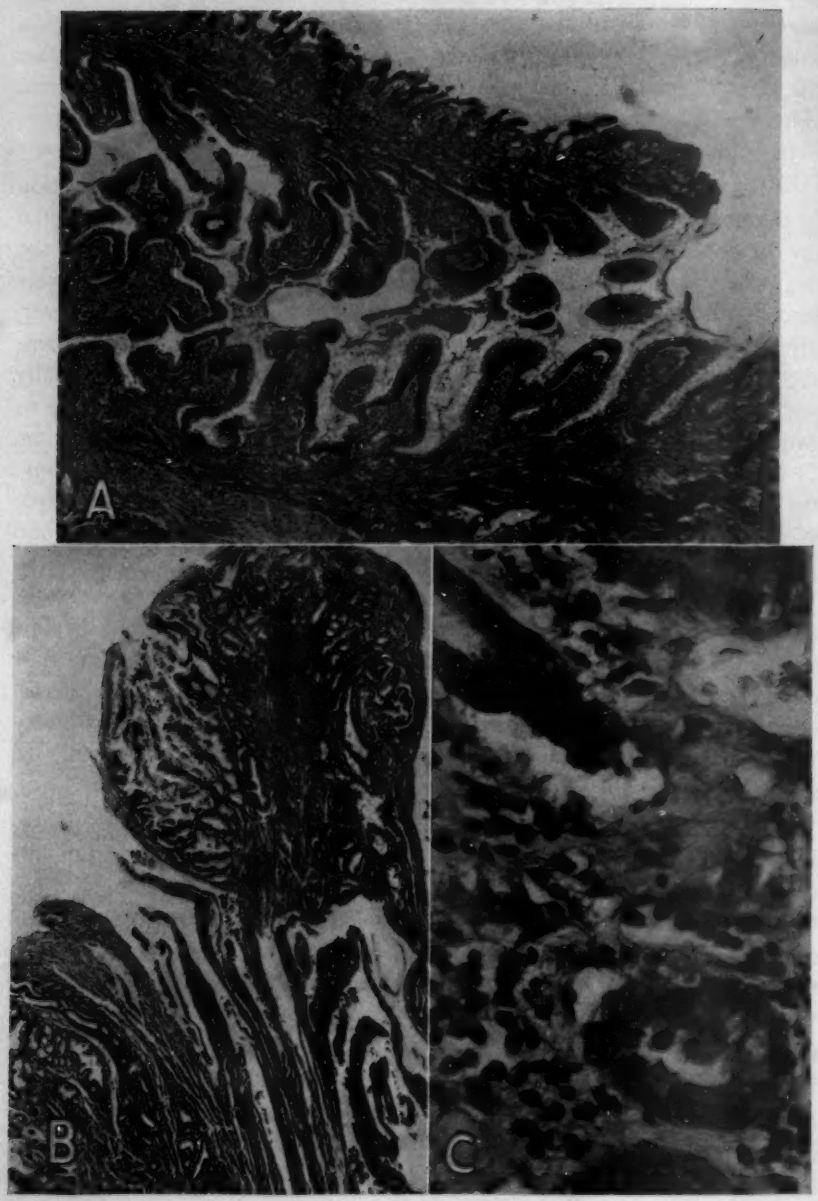


Fig. 2.—*A*, longitudinal section of the major papilla showing junction of ducts; × 42. *B*, polyp of the major papilla; × 26. *C*, adenomatoid hyperplasia in polyp, with mitosis; × 415.

### ADENOMATOID HYPERPLASIA

In the 4 cases in which a diagnosis of polyp of the papilla was made, as well as in 14 other cases of the 45 in which microscopic examination was made, there was marked proliferation of the epithelium of the papillary processes and of the wall about the orifice (fig. 2*B*). The glands were dilated and cystic, and in some glands there was an irregular arrangement of the epithelial lining, and numerous mitotic figures were present (fig. 2*C*). In some cases the nuclei were hyperchromatic and larger than normal. In relation to the nuclei the nucleoli also appeared larger than those in normal regenerating cells. In most cases hyperplasia was seen also in the stroma, and new and tortuous blood vessels and increased numbers of lymphocytes were present.

Evidence of inflammation in this series of specimens was difficult to find. Lymphocytes, indeed, often were present in large numbers, especially at the tip of the papilla. This finding cannot, however, be accepted as very strong evidence for the presence of chronic inflammation since lymphocytes are normally numerous in the duodenal mucosa. Congestion, edema and increased numbers of large mononuclear cells were prominent in 4 cases. Frank suppuration, ulceration or formation of pseudomembrane were not seen.

Analysis of the 18 cases in which adenomatoid hyperplasia was observed revealed that the mean age of the group of patients represented was 51 years while the mean age of the group in which hyperplasia was not observed was 31 years. Of the 18 cases, in 6 there were malignant tumors and in 8 benign tumors elsewhere in the body; that is, in 77.7 per cent of the cases in which abnormal hyperplasia was found, some neoplastic disease was present. Of the 27 cases in which there was no hyperplasia, in 11, that is 41 per cent, there was some associated neoplastic process. Of the 18 cases in which adenomatoid hyperplasia was a characteristic, in 2 cholelithiasis and in 2 duodenal ulcers were present. In 4 of the 27 cases in which hyperplasia was absent cholelithiasis was found, and in 3, duodenal ulcers.

### BENIGN NEOPLASMS OF THE MAJOR PAPILLA

Calzavara [1] in 1895 reported observations on 2 cases of adenoma of the papilla of Vater. In one case the lesion was a hard, fibrous growth, which had grown into the lumen without compromising it. In the other case the tumor was an adenomyoma, which gave rise to no icterus during the life of the patient although there was definite evidence of biliary stasis on examination of the liver at necropsy.

1. Calzavara, C.: Virchows Arch. f. path. Anat. **141**:221, 1895.

Upcott [2] wrote that Krause described a benign polyp in a dissertation at Kiel in 1901.

Rolleston and McNee [3] stated that the simple papillomas occasionally seen around the biliary papilla in the duodenum are growths of the intestinal surface of the papilla and are not growths of the bile duct. They also stated that papilloma may arise in the cavity of the ampulla of Vater.

Stein [4] observed a papilloma of the major papilla in examination of a patient aged 37 years. Hydrops and icterus were present. The tumor was removed through the duodenum by curettage and electrocauterization.

Pallin [5] mentioned a case observed by Forselius in which examination at necropsy revealed wartlike papillomas in the lower 2 cm. of the common duct. He also mentioned a case observed by Sundberg in which a papilloma with a long pedicle extended from the region of the papilla. Similar but smaller papillomas were found in the lower end of the common duct, which was occluded by a stone.

While discussing a paper by Bazin,[6] Archibald [7] reported the removal of a benign papilloma from the orifice of the common duct. The tumor had given symptoms of obstruction, and yet at the time of operation the common duct was dilated to only a moderate extent, and a probe could be passed easily through the duct into the duodenum. The tumor was not palpable through the wall of the duodenum and was discovered only after the duodenum had been opened.

Dardinski [8] examined the major papilla in 100 cases. In 54 he observed one or several folds of mucous membrane, polypoid in nature, protruding through and apparently obstructing the orifice. In 32 of the 54 specimens, congestion suggestive of a definite inflammatory process was observed around the orifice and occasionally in the projecting mass.

Christopher [9] reported a case in which an adenoma of the ampulla of Vater had caused obstruction of the common duct. Examination at necropsy revealed a granular nodule 5 mm. in diameter which projected into and encroached on the lumen of the common duct. Christopher mentioned 41 other instances of benign tumors of the bile ducts that

2. Upcott, H.: Ann. Surg. **56**:710, 1912.

3. Rolleston, H., and McNee, J. W.: Diseases of the Liver, Gall-Bladder and Bile Ducts, ed. 3, New York, The Macmillan Company, 1929, pp. 740-741.

4. Stein, J.: Prag. med. Wchnschr. **35**:383, 1910.

5. Pallin, G.: Beitr. z. klin. Chir. **121**:84, 1921.

6. Bazin, A. T.: Ann Surg. **92**:658, 1930.

7. Archibald, E. W., in discussion on Bazin.[6]

8. Dardinski, V. J.: Am. J. Path. **7**:519, 1931.

9. Christopher, F.: Surg., Gynec. & Obst. **56**:202, 1933.

had been reported in the literature. The ampulla had been definitely involved in only 6 of these cases.

### POLYPS OF THE PAPILLA ENCOUNTERED AT THE MAYO CLINIC

There had been 25 cases of polyp of the major papilla recorded up to Oct. 1, 1936. In no case was the diagnosis made before the patient died, and at the time of necropsy, in each case, the polyp was found as an incidental lesion. The mean age of the patients represented in this group was 55.1 years. There were 15 men and 10 women in the series. The reasons these patients gave for coming to the clinic, as well as the causes of their deaths, were varied and for the most part seemed to have no relation to the incidental finding of a polyp of the papilla. Of these patients, 6 described symptoms which might have been related to papillomas of the major papilla had there been no complicating lesions. In 3 of these patients chronic cholecystitis was present, and in 2 of these 3, cholelithiasis was present. One patient had cholelithiasis without cholecystitis. Another patient had duodenal ulcer. These findings obviously obscured any relationship that might have existed between the presence of polyps and the symptoms. They suggest, however, that inflammmation and irritation of the papillary mucosa played a part in the appearance of polypoid hyperplasia.

Grossly the polyps varied from 2 to 5 mm. in diameter and appeared usually as small, fleshy, grayish red or pink elevations that seemed to fill the orifice of the papilla. Both grossly and microscopically they resembled the papillomas described previously (figs. 2 *B* and 3 *A*).

There were few definite signs in these patients that papilloma of the papilla is of clinical significance. In 2 patients the gallbladder was so dilated that it contained more than 75 cc. of bile. In 2 others only the ducts were dilated. One of these patients had a stone in the gallbladder. One patient had dilation of the gallbladder graded 2 (on a basis of 1 to 4) and dilation of the ducts graded 3 (fig. 3 *A*). In none of these patients, however, had there been any clinical sign or symptom of obstruction.

The accumulated data suggest several etiologic factors worthy of consideration. The mean age of these patients was 55.1 years. Closely associated with the factor of increasing age was probably a decrease of normal inhibition of cellular hyperplasia or neoplasia. Thus, in 23 of the 25 patients some associated neoplastic process was present elsewhere in the body. The neoplasms of 9 patients were classified as malignant and those of 14 as benign. Also, the fact that several different types of epithelium meet at the papilla and that this region is stimulated by a variety of digestive enzymes and chemical substances deserves consideration.

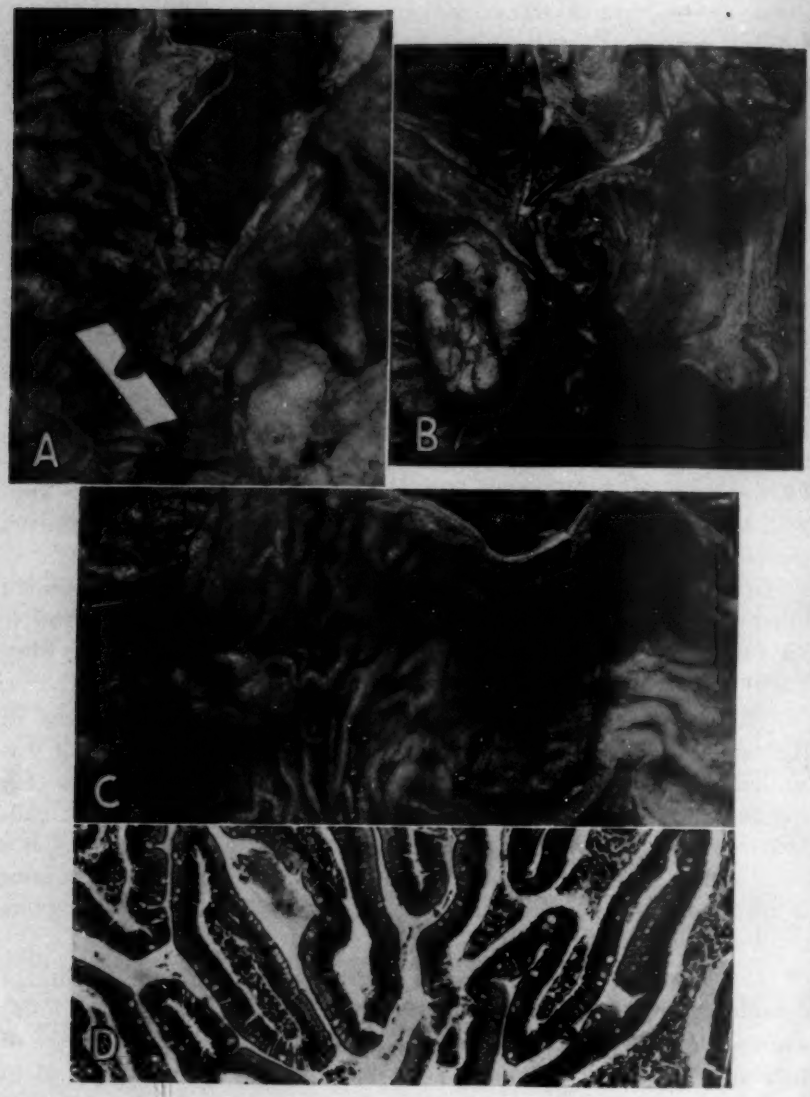


Fig. 3.—*A*, polyp of the major papilla. *B*, carcinoma of the major papilla. *C*, carcinoma of the minor papilla. *D*, adenocarcinoma (grade 1) of the minor papilla; × 110.

## INSTANCES OF CARCINOMA OF THE MAJOR PAPILLA ENCOUNTERED AT THE MAYO CLINIC

For several reasons the term "carcinoma of the papilla" is used in preference to the more frequently used expression "carcinoma of the ampulla." In the first place, an ampulla, as was shown in the early part of this investigation, is present in only about half the cases. Second, from a practical standpoint, it makes little difference from which of the many possible points of origin a neoplasm of the papilla arises. The pathologic results, symptoms and treatment are the same. In the third place, the actual identification of the site of origin of the neoplasm, even after operation or necropsy, is extremely difficult and in many cases it is impossible.

*Incidence.*—Up to Oct. 1, 1936, at the clinic, necropsy had been performed in 28 cases of primary carcinoma of the papilla of Vater. This represents an incidence of carcinoma of the major papilla of 0.21 per cent.

*Age and Sex of Patients.*—The mean age in this group was 56 years. The youngest patient was aged 34 years, and the oldest, 68 years. Three-fourths of the patients were more than 50 years old. Twenty-three (82 per cent) were men. Five (18 per cent) were women.

*Etiology.*—In the final analysis any explanation of the cause of carcinoma of the papilla in particular will depend on knowledge of the cause of carcinoma in general. This knowledge, as Borrmann-Bremen [10] pointed out, is at the present time wholly hypothetic and will not be on a firm basis of fact until more is known concerning fundamental causes of normal growth. Nevertheless, uncertain as information is in this respect, the interesting theories and facts relating to the occurrence of carcinoma of the papilla will be briefly reviewed, as this is the site of origin of duodenal carcinoma in two thirds of the cases.

Some authors believe that gallstones play a significant rôle in the causation of carcinoma of the papilla. Outerbridge,[11] however, in analyzing 110 cases reported in the literature, found that stones were present in only 23 instances (about 21 per cent). In the series reported in this paper stones were present in only 5 cases (18 per cent). Furthermore, gallstones occur more frequently among women than among men, in a ratio of 7 to 1, while carcinoma of the papilla occurs among men oftener than among women.

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10. Borrmann-Bremen, R.: Geschwülste des Magens und Duodenums, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 4, pt. 1, pp. 1004-1016.

11. Outerbridge, C. W.: Ann. Surg. **57**:402, 1913.

Many authors emphasize the hypothesis of Cohnheim,[12] namely, that carcinoma originates from embryonic rests and misplacements, but no direct evidence is available as regards the origin of carcinoma of the papilla. Evidence against this hypothesis is the finding of pancreatic tissue in 20 of 32 minor papillae, which are rarely the site of carcinoma, and the absence of pancreatic rests in the series of major papillae examined, although this structure is more frequently the site of carcinoma.

Holtbuer,[13] Geiser [14] and others have emphasized especially the fact that the papilla is a zone of transition for many types of epithelium, and they expressed the belief that this helps explain the frequent occurrence of carcinoma in this region. In regard to the bile and pancreatic ducts the epithelium of the one is so similar to that of the other in the intrapapillary portion that in most cases one cannot distinguish accurately from the character of the epithelium alone between pancreatic and bile duct. The transition between the epithelium of the ampulla and that of the duodenum is a more abrupt one, however. The fact that in many cases it was exactly in the region of this transition that hyperplasia was most marked lends support to the belief that these areas where transition occurs are sites of predilection for carcinoma.

The question of polyps and polypoid hyperplasia of the papilla in their relation to carcinoma has received scant attention from workers in this field. This is probably owing to the technical difficulties encountered in a close study of folds of the papilla. The present investigation has shown that polyps and adenomatoid hyperplasia of the papilla are not rare and that, if looked for, they prove to be comparatively frequent. The gross and the microscopic appearance of these polyps have already been described, and their similarity to low grade papillary carcinoma of the papilla is striking. It has become a well established pathologic and clinical fact that papillomas of the stomach, colon and urinary bladder are closely related to carcinomas of these organs. Some workers go a step further and call polyps in these situations carcinomas of grade 1. To consider the polyps of the papilla as inflammatory in origin need not militate against their significance. Thus Bell [15] stated, "The distinction between early stages of cancer and inflammatory hyperplasia is often arbitrary. Carcinomas of the epidermis begin as lesions of the inflammatory type and it is only when definite invasion of the underlying tissue has occurred that a certain distinction can be made. Hyperplasia of the uterine mucosa, the prostate, and the breast, pass gradually into carcinomas."

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12. Cohnheim, J.: Vorlesungen über allgemeine Pathologie: Ein Handbuch für Aerzte und Studirende, Berlin, August Hirschwald, 1882, vol. 1, pp. 732-757.

13. Holtbuer, cited by Geiser.[14]

14. Geiser, J. F.: Deutsche Ztschr. f. Chir. **86**:41, 1907.

15. Bell, E. T.: A Textbook of Pathology, ed. 2, Philadelphia, Lea & Febiger, 1934, chap. 12, p. 205.

*Gross Pathologic Change.*—The carcinomas in this series of cases were, as a rule, small growths (fig. 3 *B*). Three were small annular constricting tumors. One tumor measured scarcely 7 mm. in diameter, and yet it had caused complete obstruction at the base of the papilla. The majority of the growths were firm nodular masses, from 1 to 3 cm. in diameter, which projected into the lumen of the intestine. Six tumors were polypoid or papillomatous in character. Six had undergone ulceration. In some cases the papillomatous masses extended up the common bile duct, growing in a retrograde fashion. Grossly demonstrable invasion of the pancreas occurred in 2 cases. In some specimens it was possible to pass a probe gently through the bile duct although the clinical course indicated complete biliary obstruction. This occurrence of definite clinical obstruction without complete anatomic occlusion is difficult to explain. Possibly the neoplastic invasion of the musculature and of the nerves supplying the papilla and its ducts paralyzes the normal peristaltic action which is necessary for the passage of bile.

*Microscopic Pathologic Change.*—All the neoplasms in this series of cases were classified as adenocarcinoma, but there was a wide range in their histologic and cytologic appearance. The question as to the exact origin of each tumor could be answered only partially. It has been shown in the study of the normal papilla that the intrapapillary portions of the bile and pancreatic ducts are so similar that accurate distinction between them often is impossible. Consequently, it is at once apparent that any attempt to classify neoplasms as to origin from the epithelium of the bile duct or from that of the pancreatic duct would be fruitless. In the well differentiated tumors there was a definite similarity in the appearance of the ducts, acini and lining cells to those found in the normal papilla and a striking resemblance to those found in the papillae which had undergone polypoid hyperplasia. In 4 cases the adenocarcinoma was of grade 1, and in 4, of grade 2. In this group of cases differentiation was sufficient to suggest origin. All specimens resembled carcinoma of the ducts and possessed papillomatous processes. This indicated origin from the intrapapillary portion of the papilla. In 11 cases the carcinoma was of grade 3, and in 6, of grade 4. In this group it was impossible to trace the exact origin of the neoplasm. Practically, of course, such fine discrimination is unnecessary.

A tendency toward papillomatous formation was marked; 14 of the neoplasms showed this characteristic. Four of the tumors had produced large amounts of mucus, giving the histologic appearance of the so-called colloid carcinoma, more properly termed mucoid carcinoma.

Cellular structure varied greatly. The tall columnar cell was a constant finding in the better differentiated carcinoma of grade 1 or grade 2. Cuboidal epithelium was observed in many of the tumors, while in only 5 were the cells spheroid. Cytologic evidences of malignancy, such as

large nuclei and nucleoli, dense staining of chromatin and mitotic figures were present.

Evidence of inflammation, such as necrosis, fibrin and polymorphonuclear cells, was present in the 6 tumors that had undergone ulceration.

*Metastasis.*—Particularly illuminating from the standpoint of operative therapy is the fact that in 15 cases no evidence of metastasis was found. This substantiates the premise that these neoplasms, from a clinical standpoint, may be suspected of existing at a comparatively early period in their development. Consequently, they offer a challenge in the field of surgical treatment of internal cancer. The regional lymph nodes were involved in 10 cases, the liver in 7 and the pancreas in 5. Both regional lymph nodes and the liver were involved in 4 cases, and in 2 cases the lymph nodes, pancreas and liver all were invaded. The metastatic neoplasms corresponded closely in their degree of differentiation to the primary tumor.

*Lesions in Neighboring Organs.*—The bile ducts were dilated markedly in 23 cases. The pancreatic ducts, on the other hand, were dilated in only 13 cases. Aside from the possibility that lesser degrees of dilatation in the pancreas may not have been described, there are two other explanations of the discrepancy: (1) The firmer structure of the pancreas prevents early and marked dilatation of the ducts, or (2) the accessory duct takes over the function of the duct of Wirsung. If the latter explanation is accepted, it indicates: (1) that the accessory duct was connected to the main pancreatic duct, and (2) that the channel where the accessory duct joins the duodenum was patent.

The liver, as a rule, was enlarged, smooth and dark brownish green. Examination revealed biliary cirrhosis associated with dilatation of the large bile ducts in 10 cases, central necrosis in 10 cases and purulent cholangitis and formation of abscess in 6 cases. Marked infiltration with fat was observed in 3 cases, while chronic passive congestion was observed in 1 case and atrophy in 1 case. An increased amount of intracellular bile pigment was almost constantly found. The pigment appeared as dark brown granules of varying size, which were most numerous in the cells near the central vein. The bile canaliculi were, as a rule, markedly distended with bile. Whereas the small bile canaliculi and the large bile ducts were usually markedly distended, the ducts of intermediate size, which are surrounded by firm periportal connective tissue, rarely were dilated.

Hydrops of the gallbladder was observed at necropsy in only 9 cases. This is not remarkable when it is remembered that most of the patients had been operated on and that most of the operations included drainage of the gallbladder either externally or by gastric or duodenal anastomosis. Cholecystitis was present in 4 cases, and empyema of the gallbladder was present in 1. Suppurative pylephlebitis was found in 1 instance.

Necrosis of fat in the pancreas was observed in 4 cases, pancreatitis in 2 and interstitial edema in 1 case. There was evidence of presence of bile in the pancreatic duct in only a single case. The islands of Langerhans were notably free from evidence of pathologic change.

The cases which have just been discussed cannot, without qualification, be considered instances of primary carcinoma of the duodenum. In some cases in which the neoplasm was larger and more advanced, it is true, the possibility of an origin from the adjacent duodenal mucosa, outside the confines of the papilla, could not be ruled out. However, a neoplasm arising in the minor papilla is more properly duodenal in origin; that is, it is derived from the epithelium, ducts or the pancreatic tissue which is so frequently found in this structure. The occurrence of a neoplasm of the minor papilla is not mentioned in the literature, but undoubtedly in some of the cases of primary duodenal carcinoma reported in the literature the growth had its origin in the minor papilla. Three such cases have been observed at necropsy at the Mayo Clinic:

In the first of these cases the neoplasm was found incidentally at necropsy in the body of a man aged 43 years, who died of complications of chronic ulcerative colitis. The lesion was first diagnosed grossly as a polyp since it measured only 15 by 6 by 5 mm. (fig. 3*C*). Microscopically, however, it had the appearance of an adenocarcinoma of grade 1 (fig. 3*D*). An interesting feature of this case was the fact that the main pancreatic duct emptied through the minor papilla and was definitely dilated owing to some obstruction caused by the neoplasm.

In the second case, that of a man aged 60 years, the neoplasm had the appearance of an irregular area of ulceration extending across the first portion of the duodenum, 3 cm. from the pylorus and 1.5 cm. from the major papilla. It measured 4 by 1.5 cm. and was 1 cm. in depth. Neither the papilla nor the duct of Santorini could be identified. The growth had invaded a small portion of the head of the pancreas and obstructed the common bile duct but had not obstructed the pancreatic duct. Microscopically, it was a mucoid adenocarcinoma of grade 2, with the histologic appearance of carcinoma of the intestine rather than of carcinoma of a duct.

In the third case, that of a white man aged 50 years, the neoplasm measured 4 cm. in its greatest diameter and had invaded the pancreas. The duct of Santorini was obstructed and gave evidence of marked dilatation and of formation of cysts. Microscopic examination showed that the carcinoma had infiltrated the entire papilla without causing ulceration of the duodenal mucosa. Its general appearance suggested an origin from pancreatic ducts. The possibility that it originated from the pancreatic alveoli rather than from the ducts in the papilla must, of course, be considered.

## CLINICOPATHOLOGIC CONSIDERATIONS

The various clinical implications of the foregoing study are too numerous to allow a thorough review. It is apparent that a relatively small lesion in the papilla of Vater will give rise to early symptoms and signs because of obstruction to the flow of important secretions from

the liver and pancreas. Practically, lesions of clinical significance in this structure can be discussed under three heads: choledocholithiasis, inflammation and neoplasms.

The occurrence of gallstones impacted at the entrance to, and within, the papilla of Vater has been discussed widely. It is mentioned here only because this work presents additional anatomic evidence against the hypothesis that acute hemorrhagic pancreatitis is caused by regurgitation of bile into the pancreatic ducts. It already has been pointed out that in only 6 of 55 cases did the ducts join 5 mm. or more from the orifice of the papilla. In these cases it is conceivable that a stone might become impacted in the ampullary portion of the papilla and thus convert the bile and pancreatic ducts into a continuous channel. This would allow the possibility that bile is forced into the pancreatic ducts and causes acute hemorrhagic pancreatitis. In the other cases such an occurrence would be impossible because a stone large enough to block the bile duct would occlude simultaneously the pancreatic duct and thus effectively prevent bile from passing into it.

A point of importance to those concerned with exploratory and plastic procedures on the ducts or papilla is the extreme narrowing that takes place as the ducts pierce the duodenal wall. Often this is so pronounced that there is a definite ridge or step which partially blocks the wide extraduodenal portion of the common bile duct from the intraduodenal or intrapapillary portion. It is easy to see how when this region is explored with a probe a false passage could be forced unless care and gentleness were employed.

Inflammation of the papilla in conjunction with gastroduodenitis and general cholangitis since the time of Virchow has been considered important in the pathogenesis of catarrhal jaundice. More recently the view that this symptom complex is primarily owing to hepatitis has been gaining favor. Many workers are convinced, however, that catarrhal jaundice is not an entity either as to causes or as to pathogenesis. Inflammation of the papilla with congestion, edema, excessive secretion of mucus and either partial or complete obstruction of the ducts undoubtedly plays a role in some types of jaundice. Evidence obtained at postmortem examination to support this point of view is lacking because patients who have this disease almost always recover.

Inflammation of the papilla, aside from the probable role it plays in catarrhal jaundice, has received scant attention as a cause of symptoms. The reason for this lies in the fact that obstruction of the ducts, when it occurs, is incomplete and transitory and usually ceases to exist before jaundice appears clinically. As has been shown experimentally, complete obstruction must exist for several days before jaundice appears clinically. Even though jaundice does not appear, it is nevertheless held likely that inflammation of the papilla can and does cause symptoms.

How often inflammation occurs in this structure and how it can be recognized clinically cannot be determined at the present time. Records of necropsy are of slight help in solving this problem because the lesion as such does not cause death. The anatomic diagnosis of edema of the papilla of Vater was made only twice, according to the records of necropsies at this clinic. In one case it was associated with hydrohepatosis and cholelithiasis. In the other case there was also chronic cholecystitis, cholelithiasis, choledocholithiasis and ulceration of the common bile duct. Obviously, these instances are too few and too complicated to be of value for purposes of deduction. The 2 cases in which there was duodenal ulcer at the papilla of Vater which MacCarty[16] reported in 1910 are also interesting in this connection and demonstrate the results of severe inflammation in this structure.

On the whole, it is probable that the ability of uncomplicated papillitis to produce symptoms is underestimated. Such a condition should at least be considered as a factor in actual catarrhal jaundice, in symptoms occurring after cholecystectomy and in obscure gastrointestinal symptoms when organic disease of other organs has been ruled out.

The clinical significance of malignant neoplasms of the papilla is obvious and undisputed. Polyps and adenomatoid hyperplasia, the significance of which is not as obvious, are nevertheless felt to be of considerable importance, first, as a cause of obstruction, especially when associated with inflammation, and, second, because of their relationship to carcinoma arising in this structure.

## SUMMARY

In an investigation of the anatomic and histologic characteristics of the major duodenal papilla in 100 cases there were many observations of pathologic interest. Among the more significant of these were the following: 1. A junction of bile duct and pancreatic duct occurred in 33 of 55 specimens examined. In only 6 of these specimens was the ampulla 5 mm. or more in length. 2. There was marked narrowing of the bile and pancreatic ducts as they pierced the duodenal wall, and this narrowing was increased by the presence of mucosal folds in the intraduodenal portions. 3. Polyps of the papilla were found in 4 of 100 postmortem examinations in which special search was made for them.

Lesions of the major papilla which were observed in routine postmortem examinations at the clinic up to Oct. 1, 1936, also are described. These include 2 examples of ulceration, 2 of edema, 25 of polyp and

16. MacCarty, W. C.: The Pathology of the Gall-Bladder and Some Associated Lesions: A Study of Specimens from Three Hundred and Sixty-Five Cholecystectomies, in Collected Papers by the Staff of St. Mary's Hospital, Mayo Clinic [1910], Philadelphia, W. B. Saunders Company, 1911, vol. 2, p. 151.

28 of carcinoma (0.21 per cent). In addition to these there were 3 examples of carcinoma of the minor papilla, which are considered briefly.

Because of the structural peculiarities of the major papilla, inflammation of this structure may give rise to symptoms and sometimes will produce jaundice. Polyps of the papilla are clinically of some significance, because, first, they may produce obstruction of themselves and, second, because they probably are in close relationship to carcinoma.

# Case Reports

## CYSTIC LYMPHANGIECTASIA OF THE ADRENAL

S. Milton Rabson, M.D., New York, and Edward F. Zimmerman, M.D., Wichita, Kan.

The relative infrequency of cysts and the rarity of vascular hyperplastic and neoplastic formations in the adrenal gland prompt the report of this case.

### REPORT OF CASE

A white man of 65 years died of cardiac failure two days after cholecystectomy and appendectomy. At necropsy, twelve hours later, generalized marked atherosclerosis was observed in the aorta, coronary vessels and renal vessels. The heart was moderately hypertrophied (400 Gm.).

The right adrenal was normal. The left adrenal surmounted the upper pole of the kidney, on its concave border. It was embedded in much fat and was distinctly enlarged, particularly in its central portion. The gland measured 7 cm. in length and had a maximum width of 2.7 cm. and a thickness of 2 cm. Freed of fat, it weighed 11 Gm.

In the midportion, where the thickness was maximal, there were distinct round cystic areas elevating the surface. These had transparent delicate gray walls, through which the watery clear content was easily recognized. On section the central prominence was seen to be produced by a pearly gray mass with indefinite borders (fig. 1). Throughout this mass there were numerous spaces and cysts. The largest cyst was 1.2 cm. in diameter, and its content was viscid. There were few cysts, however, of such size. Most of the spaces did not reach a diameter of 1 mm. and were barely discernible with the unaided eye as pinpoint pits in the solid tissues.

The largest cyst was centrally placed midway between two adrenal surfaces. It was lined by an irregularly thick wall of poorly cellular, moderately dense hyalinized connective tissue. Where the wall was thickest the outer lamellae were densely infiltrated by lymphocytes. Where the wall was thinnest there were openings leading to and from adjacent confluent cystic spaces (fig. 2*a*). The latter were lined in some places by a single layer of flattened spindle cells, endothelium. Other spaces had walls of connective tissue and lacked endothelial linings. Elastic fibers were not recognized. The spaces were partly or completely filled with a fine network of coagulated eosinophilic material.

The cystic structures elevated the slightly thickened adrenal capsule and even disrupted it to extend outward into the periadrenal fat. Adjacent to these extensions there were numerous congested capillaries and arterioles in the fat, which was in large part replaced by lymphoid tissue. True follicle formation, however, was not recognized.

The spaces and cysts extended laterally into the normal adrenal tissues. The process began in the medulla and spread into the cortex in wedgelike fashion,

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with apex outward. At the apex there were one or two small distinct spaces, while at the base, in the medulla, there were as many as twenty confluent cavities. Between the cysts compressed, atrophying parenchyma, in part of medullary character, was easily recognized (fig. 2*b*). It was evident that the cystic spaces arose between the epithelial cords and not from a degeneration or necrosis of epi-

Fig. 1.—Cystic lymphangiectasia of an adrenal, showing large cysts and numerous minute cysts (pinpoint depressions in the solid tissue); × 2.

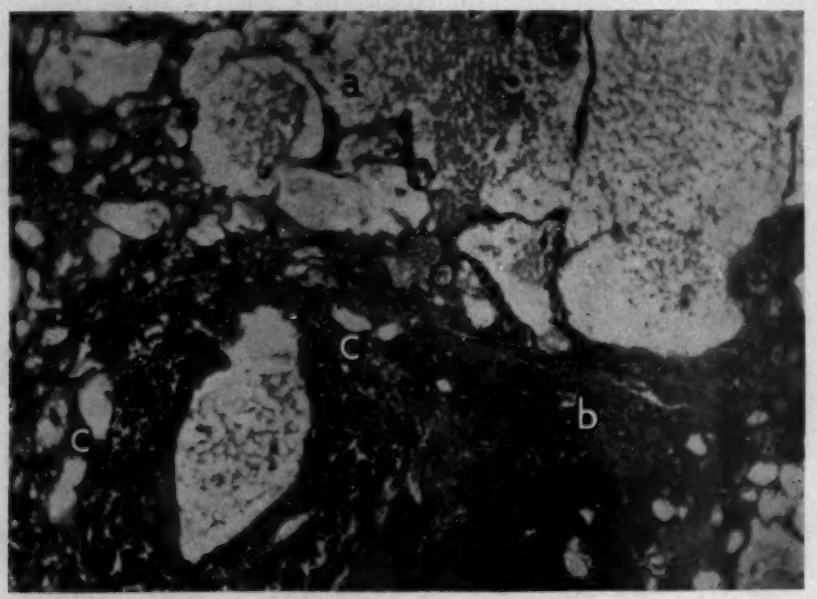


Fig. 2.—Confluent lymph spaces and cysts (*a*), with intact intervening adrenal parenchyma (*b*), and dilatation of perivenous lymph spaces, with accompanying lymphocytic infiltration (*c*); × 80.

thelial cells. Accompanying the cystic process was a thickening of the normally delicate collagen fibers between the adrenal cortical islands, to aid in forming the walls of the new spaces.

The larger vessels of the adrenal showed no striking changes. Apart from slight eccentric thickening of the intima of a few veins, the vessels were unaltered.

There was distinct dilatation of the lymph spaces in the venous adventitia, which were continuous with the smaller adjacent cysts (fig. 2*c*). The gross and microscopic observations suggested a diagnosis of cystic lymphangiectasia of the adrenal.

Although lymph vessels have been described in the adrenal cortex in horses, calves and cattle,[1] such channels have been found in man only in the capsule and in the medulla.[2] The finding, therefore, of lymph spaces in the medullary area, with distinct walls of endothelium and with or without connective tissue, is not unexpected. Such formations in the cortex are necessarily of different genesis. Fetal malformation or a hamartoma is readily suggested,[3] in which lymph vessels have been formed in marked excess without complete linkage with the rest of the lymph vessel apparatus, the condition being aggravated by cardiovascular stasis.[4] Most of the two dozen cases reported fall into the group of hamartoma associated with cystic dilatation.[5]

In the reported cases the cysts have varied in diameter from a fraction of a millimeter to 13 cm. One case has been recorded in which the cyst contents totaled 5 liters.[5a] There have been twice as many cases in women as in men. Most of the patients were between 31 and 70 years old, with 7 in the fourth decade, 4 each in the next two decades and 3 in the seventh decade of life. One case has been reported as occurring before the age of 5 years, another between 11 and 15 years of age and another in the third decade. Bilateral cysts have been half as common as those occurring only in the right or the left adrenal. In some cases only were diagnoses made, of cystic lymphan-

1. Stilling, H.: Virchows Arch. f. path. Anat. **109**:324, 1887.

2. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1935, p. 311. Schaffer, J.: Lehrbuch der Histologie und Histogenese, ed. 3, Leipzig, Wilhelm Engelmann, 1933, p. 326.

3. (*a*) Borst, M.: Echte Geschwülste (Gewächse, Blastome), in Aschoff, L.: Pathologische Anatomie, ed. 7, Jena, Gustav Fischer, 1928, vol. 1, p. 689. (*b*) Bossard, R.: Ein Fall von Lymphangioma cysticum der rechten Nebenniere, Zurich, Emil Cotti's Wwe., 1900. (*c*) Riess, P., and Schott, E.: München. med. Wchnschr. **76**:621, 1929. (*d*) Sézary, A., and Hirschberg, F.: Bull. et mém. Soc. anat. de Paris **9**:150, 1923. (*e*) Terrier, F., and Lecène, P.: Rev. de chir., Paris **34**:321, 1906. (*f*) Winter, H.: Zentralbl. f. allg. Path. u. path. Anat. **63**:305, 1935. (*g*) Henschen, K.: Beitr. z. klin. Chir. **49**:217, 1906.

4. Beck, M.: Zentralbl. f. allg. Path. u. path. Anat. **47**:181, 1929.

5. (*a*) Bourcy, P., and Legueu, F.: J. d'urol. **1**:181, 1912. (*b*) de Vecchi, B.: Virchows Arch. f. path. Anat. **200**:151, 1910. (*c*) Dietrich, A., and Siegmund, H.: Die Nebenniere und das chromaffine System, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 1050. (*d*) Lubarsch, O.: Zystisches Lymphangiom der Nebenniere (illustration in Dietrich and Siegmund[5c]). (*e*) Manca, C.: Arch. ital. di anat. e istol. pat. **3**:553, 1932. (*f*) Montaldo, G.: Tumori **10**:447, 1936. (*g*) Nowicki, W.: Virchows Arch. f. path. Anat. **207**: 338, 1912. (*h*) Oberndorfer, S.: Beitr. z. path. Anat. u. z. allg. Path. **29**:516, 1901. (*i*) Preusse, O.: Zentralbl. f. allg. Path. u. path. Anat. **25**:961, 1914. (*j*) Potherat and Chambord, cited by Brin, M. H.: Assoc. franç. d'urol., Proc.-verb. **15**:33, 1911. Bossard.[3b] Riess and Schott.[3c] Sézary and Hirschberg.[3d] Terrier and Lecène.[3e] Beck.[4]

gioma,[6] lymph cyst,[6a] serous cyst [7] and adrenal cyst,[8] the latter in children under 5 years.

The walls of the spaces and cysts have been described as formed of endothelium,[9] of connective tissue [5i] or of both [10] as in our case. We were unable to find elastica tissue,[11] smooth muscle,[12] nerves [5a] or areas of calcification [13] in the walls, as others have reported. Attention has been called to infiltrates of "small cells" [14] in and about the cysts, described by Dietrich and Siegmund [5c] as not differing from sympathogonia. In our case the cells were definitely lymphocytes, both free and in lymphoid tissue (fig. *2 c*). Thickening of the walls of adjacent medullary vessels, referred to by others,[15] was present in our case only to a slight degree, in a vein.

True lymphendothelial neoplasms have been less frequently encountered, both benign [16] and malignant.[17] Hemendotheliomas have been reported rarely. These included "cavernous hemangiomas" [18] and malignant hemendotheliomas or hemangiomas.[19] One case has been recorded [4] in which, besides the lymphangioma, there was another cyst, lined by ciliated epithelium, probably from aberrant gut or from kidney anlages. The absence of proliferating endothelium excludes a diagnosis of a true neoplasm (lymphangioma), a diagnosis too often used in cases of hamartoma (cystic lymphangiectasia).

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6. Goldzieher, M. A.: The Adrenals, New York, The Macmillan Company, 1929, p. 195; Die Nebennieren, Wiesbaden, J. F. Bergmann, 1911, p. 78.

6a. Ogle, J. W.: Tr. Path. Soc. London **16**:252, 1865.

7. Wallmann, H.: Ztschr. d. k.-k. Gesellsch. d. Aerzte zu Wien **16**:785, 1860.

8. Campbell, M. F.: Pediatric Urology, New York, The Macmillan Company, 1937, vol. 2, pp. 291 and 300.

9. Bossard.[3b] Sézary and Hirschberg.[3d] Bourcy and Legueu.[5a] Oberndorfer.[5h]

10. Riess and Schott.[3c] Terrier and Lecène.[3e] Winter.[3f] Beck.[4] de Vecchi.[5b] Montaldo.[5f]

11. Sick, K.: Virchows Arch. f. path. Anat. **172**:445, 1903. de Vecchi.[5b] Oberndorfer.[5h] Potherat and Chambord.[5j]

12. Beck.[4] de Vecchi.[5b] Potherat and Chambord.[5j]

13. Lucksch, F.: Beitr. z. path. Anat. u. z. allg. Path. **62**:204, 1916. Bossard.[3b] Riess and Schott.[3c] Winter.[3f] de Vecchi.[5b]

14. Beck.[4] de Vecchi.[5b] Potherat and Chambord.[5j]

15. Beck.[4] de Vecchi.[5b] Manca.[5e] Nowicki.[5g]

16. Menon, T. B., and Annamalai, D. R.: J. Path. & Bact. **39**:591, 1934. Sick.[11] Lucksch.[13]

17. Pepere, A.: Ghiandole a secrezione interna, in Foà, P.: Trattato di anatomia patologica, Turin, Unione tipigrafico-editrice torinese, 1922, vol. 8, p. 172.

18. Müller-Stüller, M., Jr.: Virchows Arch. f. path. Anat. **290**:177, 1933. Schmieden, V., and Peiper, H.: Arch. f. klin. Chir. **143**:490, 1926. Schwarz, J.: Beiträge zur speziellen Pathologie der Nebennieren, Inaug. Dissert., Munich, Müller & Steinicke, 1912.

19. Halbfass-Ney, P.: Frankfurt. Ztschr. f. Path. **37**:468, 1929. Klebs, E.: Handbuch der pathologischen Anatomie, Berlin, A. Hirschwald, 1876, vol. 1, pt. 2, p. 579; 1869, vol. 1, pt. 1, p. 471. Rabson, S. M.: Arch. Path. **25**:185, 1938. Sotti, G.: Arch. de méd. expér. et d'anat. path. **22**:762, 1910. Tavernari, A.: Arch. ital. di urol. **13**:481, 1936. Volkmuth, W.: Ueber Angiome der Nebennieren, Inaug. Dissert., Bonn, 1919. Campbell.[8]

# GENERALIZED ESSENTIAL XANTHOMATOSIS (TYPE NIEMANN-PICK) ASSOCIATED WITH PRIMARY CARCINOMA OF THE LIVER IN AN INFANT

HAROLD WOOD, M.D., CHARLESTON, S. C.

The Niemann-Pick type of xanthomatosis has been variously named. According to Abt and Bloom,[1] it was first described by Niemann, in 1914; in 1916 it was designated by Knox, Wohl and Schmeisser as a juvenile form of Gaucher's disease, and in 1924 it was separated by Pick as a clinical and pathologic entity. Bloom's [2] first paper on the disease was published in 1925, and he designated the condition as essential lipoid histiocytosis. This term was based on observations indicating that the cells primarily involved in the disease are the histiocytes, although in a later paper, on the histogenesis of the disease, Bloom,[3] clearly stated that other types of cells were involved as well. In 1932 Pick [4] reviewed the forms of xanthomatosis in the Dunham Lectures at the Harvard Medical School. He criticized Bloom's term on the ground that the disease is by no means limited to the histiocytes but involves framework and even parenchymatous cells. He preferred to designate the disease as a form of generalized essential xanthomatosis until a more satisfactory term, preferably one based on etiology, could be deduced.

The generalized essential xanthomatosis of Niemann and Pick is a rare disease. Pick [4b] stated that up to 1932 a few more than 15 cases had been reported. Since then there have been listed in the *Quarterly Cumulative Index Medicus* reports on approximately 11 more cases of this disease. Instances of such a rare disease are in themselves worth reporting, but the case described here is unique in that there was also present a typical primary carcinoma of the liver. The tumor was recognized as such at autopsy, but it was not until the microscopic examination of the sections that an abnormal storage of lipoid was suspected. Primary carcinoma of the liver is relatively rare in infants and children but is probably the most common carcinoma found in infants. In connection with an unfinished paper on the subject, I have collected reports of slightly over 100 cases published since 1841. The present case is the second in infants on whom I have made autopsies.

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From the Department of Pathology, the Medical College of the State of South Carolina.

1. Abt, A. F., and Bloom, W.: J. A. M. A. **90**:2076, 1928.
2. Bloom, W.: Am. J. Path. **1**:595, 1925.
3. Bloom, W.: Arch. Path. **6**:827, 1928.
4. Pick, L.: (*a*) Am. J. M. Sc. **185**:453; (*b*) 601, 1933.

### REPORT OF CASE [5]

A white girl 8 months old, of German parentage, was admitted to the Roper Hospital March 21, 1936. One week before, the mother, while bathing her, noticed a hard mass in the right upper portion of the abdomen. The family physician was consulted, and on his advice the infant was brought to the hospital. Previous to this the health of the infant had been unimpaired.

The infant was well nourished, well developed and strong. The temperature was 99 F., and the skin was warm and elastic. There were no abnormal cardiac or pulmonary signs. In the abdomen was a firm mass, unattached to the wall, extending from the right costal margin to the umbilicus and from the midline into the right flank. Because of its position, the mass was believed to be connected with the right kidney. The external genitalia and the extremities appeared normal. The clinical impression was that of hypernephroma.

An intravenous pyelogram made March 22 was interpreted by Dr. Hillyer Rudisill Jr. as showing incomplete filling of the renal pelves, but the pelves were located, and the outlines of the kidneys were intensified. On the right side was a mass, continuous with the liver, above; it had displaced the right kidney medially and compressed the kidney. There was no suggestion of lesions related to the left kidney.

Except for a trace of albumin in the urine and a hemoglobin content of 68 per cent, there were no pertinent laboratory data.

March 24 an exploratory laporotomy was made by Dr. F. G. Cain. He described a highly vascular bluish red tumor which occupied the medial two thirds of the right lobe of the liver, increasing the size of the organ to about three times the normal. Because of the vascularity, no biopsy was attempted. The postoperative opinion was that this was "probably primary sarcoma of the liver."

Recovery from the operation was rapid and uneventful, and the infant was discharged on the sixth postoperative day. She remained at home, in a neighboring town, where she became progressively emaciated, and the tumor mass rapidly enlarged. Death occurred September 19 approximately six months after the patient's admission to the hospital, at the age of 14 months.

*Autopsy.*—The body was that of an extremely emaciated white girl. The face had a drawn, "old" appearance. The scleras had no yellow tinge, and the skin was white, dry and stretched over the bony prominences. Distention of the abdomen was produced by a firm mass, palpable in the superior portion. A thin longitudinal scar, 6 cm. in length, was present over the superior portion of the right rectus abdominalis muscle, and in the underlying subcutaneous tissue there were prominent veins. The external genitalia had no unusual appearance. Midline section of the thoracic and abdominal walls anteriorly revealed practically no subcutaneous fat, and the pectoral and abdominal muscles were thin and pale.

A tumor mass arising from the liver occupied the right and most of the left superior portion of the peritoneal cavity and displaced the stomach and intestines inferiorly, posteriorly and to the left. The mass extended well below the level of the umbilicus, and anteriorly was attached by vascular adhesions to the abdominal wall beneath the surface scar. There was no excess of fluid in the peritoneal cavity, and the peritoneal surfaces were smooth and gray.

When the pleural cavities were opened, the lungs collapsed as usual, and the cavities contained no fluid and no adhesions. The pericardial cavity was lined with a moist serous membrane and contained no excess of fluid. The thymus gland was so small as to be almost indistinguishable.

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5. Dr. Frank G. Cain gave me permission to use the clinical data on this case.

The firm, finely grained myocardium was pale, but the chambers of the heart were not dilated. The valve leaflets and cusps were thin and flexible, the coronary ostia were patent, the ductus arteriosus was obliterated, and there was a thin diagonal slit representing the foramen ovale, considered to be functionally closed.

The lungs were crepitant and soft, and the usual lobar division was present. Bulging beneath the pleura of the anterior inferior portion of the right upper lobe were two reddish gray tumor nodules, the larger 1 cm. and the smaller 2 mm. in diameter. A similar nodule, 1 cm. in diameter, was present in the same region of the left upper lobe. The bronchi and the pulmonary arteries were patent, and the bronchial lymph nodes were small and firm.

The spleen appeared slightly enlarged, but the capsule was smooth, the malpighian corpuscles were clearly visible, and the pulp was firm and dark red.

The mucosa of the digestive tract was intact throughout, and there were no gross lesions of the wall.

All the mesenteric lymph nodes were small, pale tan and firm.

The liver weighed 750 Gm. (after fixation). Arising from the inferior portion of the right lobe and the inferior medial portion of the left lobe was a tumor mass 11 by 11 by 8 cm. Superiorly it was well demarcated from the liver but not encapsulated, and inferiorly the downward bulging mass was apparently covered with peritoneum. The surface of the growth was bosselated, and the mass had in general the shape of a slightly compressed sphere. The consistence of the tumor was more soft than normal liver, particularly in the central region, where there were irregular areas of hemorrhage and necrosis which obscured the characteristics of the tissue. The better preserved tumor tissue was gray and friable and arranged in indefinitely distinct nodules of varying size, which tended to coalesce. At the junction of liver and tumor there was evidence of compression of the liver tissue, and in this zone there were small tumor nodules separate from the main mass (fig. 1*A*). The external and cut surfaces of the remainder of the liver showed no distinct alterations except pallor, and the lobular markings were faintly visible. The gallbladder and the cystic and common bile ducts were stretched over the posterior surface of the tumor. The hepatic duct was attached at the hilus of the liver just superior to the tumor, and from that point the right and left hepatic ducts could be traced to the respective lobes in the intact liver tissue. The extrahepatic biliary tract was patent. No extension of the tumor into the inferior vena cava was present.

The kidneys were of average size for the age, the cortical surfaces were smooth, and the capsules were thin. The cortical and medullary markings were prominent, the pyramids were well formed, and the pelves and ureters were not dilated. The bladder contained a small amount of urine. The uterus, tubes and ovaries were of average size for the age and showed no gross abnormalities.

The size and configuration of the adrenals were as usual. The cortex was thin and pale yellow and the medulla indistinct.

The pancreas was firm and lobulated and the pancreatic duct patent.

The form of the consent for an autopsy did not warrant removal of the organs of the neck or examination of the cranial cavity.

*Histologic Observations Referable to Lipoid Storage Disease.*—The splenic pulp was replaced by large polyhedral mononuclear cells with centrally situated compact dark nuclei and cytoplasm of a foamy appearance and showing a finely granular acidophilic reticulum (fig. 2*A*). In paraffin sections stained with hematoxylin and eosin the foamy and reticular appearance was absent in many of the cells, and the cytoplasm appeared "empty." These cells were distributed diffusely

throughout the sections; they displaced lymphocytes at the periphery of the small malpighian corpuscles but were not present in the capsule, trabeculae or walls of blood vessels. In spite of the close packing of the foam cells, the splenic sinuses were unusually large and appeared as extremely thin-walled channels lined with elongated flat cells, comparable to vascular endothelial cells (fig. 2 *B*). Most

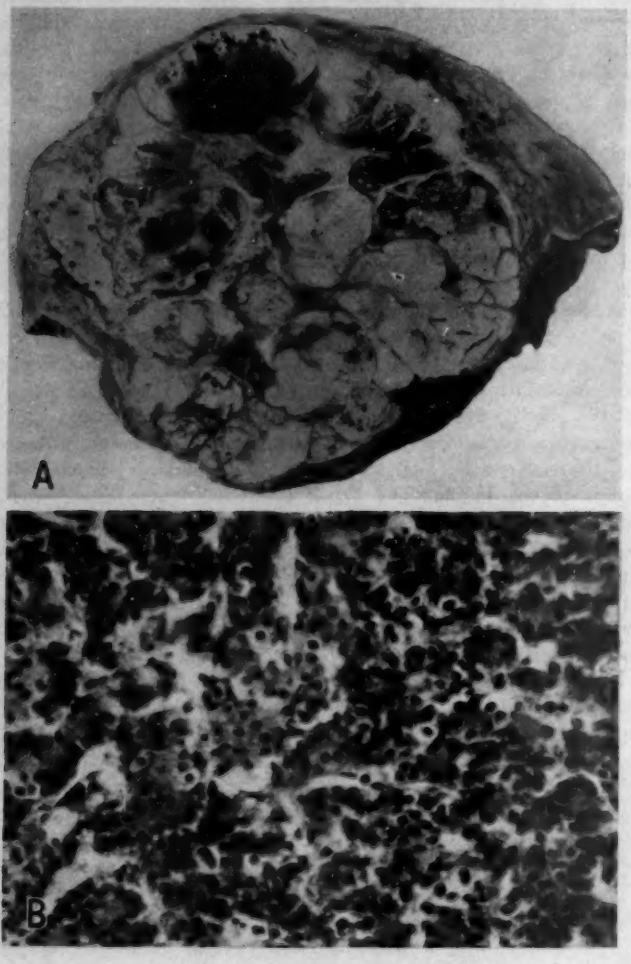


Fig. 1.—*A*, the sectioned surface of the carcinoma of the liver, showing the nodular configuration, the dark areas of hemorrhage and the gray areas of necrosis. The specimen is shown about ⅛ of the actual size. *B*, a section of the carcinoma of the liver, illustrating the cell type and the cellular arrangement; hematoxylin and eosin stain; × 250.

of these sinuses were flattened rather than round or oval and were more clearly seen in sections stained by Foote's [5a] method for demonstrating reticular fibrils.

5a. Foote, N. C.: J. Lab. & Clin. Med. **9**:777, 1924.

The cells lining the sinuses had a scant amount of cytoplasm, which exhibited no foamy or vacuolar appearance, and these lining cells were not definitely associated with reticular fibrils. Reticular cells were readily identified by their close association with the reticular fibrils, and cells so identified had no foamy or vacuolated appearance of the cytoplasm. The reticular fibrils, however, lay among the foam

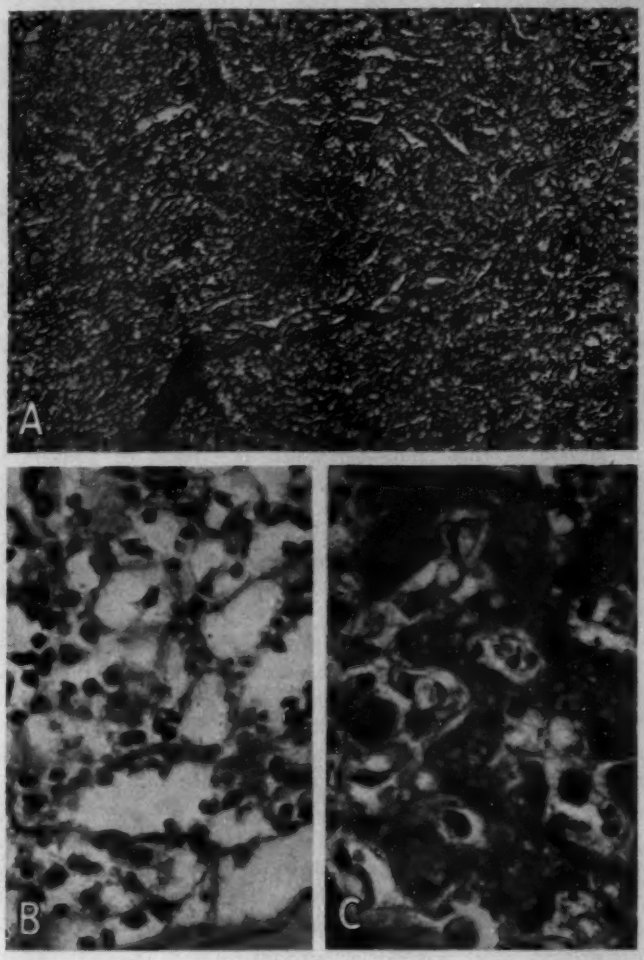


Fig. 2.—*A*, low power magnification of a section of the spleen, showing the prominent sinusoids and the replacement of the pulp by foam cells, which permeate the periphery of the malpighian corpuscle; hematoxylin and eosin stain; × 46.4. *B*, high power photomicrograph of a section of spleen, showing the foam cell characteristics and the flat endothelial-like cells which line a sinusoid, situated in the lower central portion of the field; hematoxylin and eosin stain; × 522.5. *C*, high power photomicrograph of a section of liver, well removed from the tumor, showing several elongated cells containing cytoplasmic vacuoles, situated between the liver cell trabeculae; hematoxylin and eosin stain; × 522.5.

cells in such close relation, some even appearing to pass through the foamy cytoplasm, that it seemed probable that many of the foam cells were derived from reticular cells.

In the liver, but not in the tumor, there were large and small cells with foamy or vacuolated cytoplasm situated between the cords of liver cells (fig. 2*C*). Sections stained with hematoxylin and eosin, as well as those stained by Foote's method for demonstrating reticular apparatus, showed such cells projecting into the sinusoids and between the sinusoid walls and the liver cells. While many of these cells were large and polyhedral, others were small, oval, elongated or branching, with incomplete replacement of the cytoplasm by the foamy material. These observations suggested that the involved cells represented both Kupffer cells and endothelial cells of the sinusoids. A few of the liver cells contained fine cytoplasmic vacuoles. No foam cells were present in the portal areas. In general, the spaces between liver cell cords were wider than usual, the sinusoids were not distinct, and with low or medium power magnification these regions had a fuzzy appearance.

The lung sections contained tumor nodules and small patches of acute pneumonic exudate. In areas of hemorrhage surrounding the tumor nodules and in connective tissue trabeculae within the tumor tissue there were numerous typical foam cells. Pulmonary tissue unobscured by tumor and exudate contained foam cells, singly or in small groups, situated within alveolar walls or bulging from the alveolar lining, but only rarely was such a cell seen free in an alveolus.

The one section of small intestine, which was taken at random, exhibited conspicuous dilatation of lymphatics in the submucosa and serosa and to a less degree in the mucosal villi. Some of these spaces contained a few small round cells, probably lymphocytes, and a fine acidophilic reticulum, and one contained a foam cell. Grouped around the latter lymphatic, which was in the serosa, were clumps of foam cells. In the section of large intestine there was no dilatation of lymphatics, and foam cells were absent.

Lymph nodes from the mesentery and from the region of the gallbladder were small and compact, and although the sinuses of those from the former were slightly prominent, they would probably have caused no comment except for the prominence of the lymphatics of the small intestine. No foam cells were present in these nodes, but in a small lymph node adjacent to the thymus a few cells with foamy cytoplasm were scattered about in the reticulum.

The lobules of the thymus gland were extremely atrophic and were widely separated by connective tissue. Within the lobules themselves there were many collagenous fibrils as well as reticular fibrils, and the lymphocytic element was dispersed in this framework. Scattered about were large mononuclear cells, about the size of the foam cells seen elsewhere but with faintly eosinophilic, finely granular cytoplasm. These granules did not give the prussian blue reaction for iron pigment. Most of the thymic corpuscles were reduced to granular bodies with a mixed acidophilic and basophilic standing reaction, but a few contained fragments of the hyaline laminated material usually present.

*Histochemical Staining Reactions.*—The results of some of the methods for demonstrating lipoids were not satisfactory, because by the time the lipoid storage disease was suspected all of the splenic tissue saved had been stored in waste alcohol (about 80 per cent) for several months and much of the lipoid substance had been removed. The liver was preserved during that period in Kaiserling's

solution 1,[6] and all staining methods employed were carried out on sections from that liver tissue as well as on sections of the spleen.

The content of the foam cells in the liver stained brilliantly with sudan 3 and scarlet red, both in unmordanted frozen sections and in frozen sections mordanted for several hours in concentrated potassium dichromate. Many of the foam cells of the spleen stained similarly, but some retained the same "empty" appearance of the cytoplasm as described in the routine paraffin sections. The lipoid cytoplasmic content appeared as large and small globules. The vacuoles described in the liver cells in the paraffin sections appeared as lipoid globules with these stains. In the spleen there were large extracellular collections of lipoid globules beside trabeculae and large blood vessels as well as separate from these structures.

In the spleen there was no reaction with nile blue sulfate in either mordanted or unmordanted sections. Mordanted sections of the liver, however, contained some areas in which the vacuolated cells showed a faint lavender coloration. This reaction did not appear until several minutes after the sections had been mounted in a glycerin and gelatin mixture. The dye tended to diffuse in the mounting mediums and seemed to have an affinity for the foam cell cytoplasm.

The Lorrain Smith-Dietrich[7] method for lipoids was not successful with splenic tissue, but with the liver it revealed the foam cell cytoplasm composed of dark gray, almost black, droplets.

Sections of liver and spleen stained with Mallory's aniline blue showed none of the wavy striation of the foam cell cytoplasm said to be characteristic for Gaucher's cells.

The prussian blue reaction for iron pigment was not positive in the foam cells of the liver and spleen, but the liver cells contained a few fine granules which stained dark blue.

*Histologic Observations on the Tumor of the Liver.*—The tumor (fig. 1*B*) was divided into large and small nodules by connective tissue trabeculae, which contained the larger blood vessels, and it was characterized by its extreme cellularity and the decided tendency of the cells to resemble liver cells and to be arranged in narrow cords and small clumps. The cords and clumps of cells were separated by fine capillaries. The architecture was thus suggestive of the structure of liver tissue, although there was no suggestion of true lobular arrangement, and there were no bile ducts. Canaliculi filled with pale green material resembling bile were present, situated between adjacent tumor cells. The cells of the tumor were well differentiated, although the nuclei were of a primitive type; mitoses were present, but amitotic division was not conclusively demonstrated. In general, the cell shape was polyhedral, the size was comparable to the size of the liver cells, and the nuclei were rather large and vesicular, with a large nucleolus in each. The cytoplasm was finely granular but had a decided basophilic tendency, unlike the "normal" liver cells. Some of the clumps of tumor cells had a circular arrangement, with peripherally placed nuclei, and in the centers of some of these clumps were canaliculi with bilelike content. The whole structure was built on a delicate framework of reticulum and collagen fibrils with a prominent capillary bed. In the more central portions there were large and small areas of hemorrhage and necrosis.

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6. Kaiserling's solution is made up of the following constituents: formaldehyde, 200 cc.; water, 1,000 cc.; potassium nitrate, 11 Gm., and potassium acetate, 21 Gm.

7. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924, p. 187.

Large veins at the periphery of the tumor were wholly or partially occupied by typical tumor tissue. There was evidence of centrifugal pressure from the tumor in that the architecture of the liver in a zone about 1 cm. wide surrounding the tumor was altered. The liver cords were somewhat broken up and tended to conform to the outline of the tumor surface. The liver cells were in all stages of compression atrophy, and there was either increase in or condensation of the stroma, so that it formed a dense zone adjacent to the tumor. In some of the larger connective tissue trabeculae extending into the tumor, atrophied liver cells persisted. The outer portion of this zone merged with the liver tissue beyond, which was unaltered except for the features of the lipoid storage disease.

The tumor nodules in the lungs conformed morphologically to the main hepatic tumor and contained areas of hemorrhage. Several small branches of the pulmonary arteries were plugged with tumor tissue, but there was no evidence of infarction.

*Histologic Observations on Other Structures.*—Sections of the heart, pancreas, kidneys, adrenal glands, bladder, uterus, fallopian tube and ovary showed no significant alterations.

## COMMENT

This case is presented as one of generalized essential xanthomatosis (type Niemann-Pick) associated with primary carcinoma of the liver, hepatic cell type, and with terminal emaciation and acute lobular pneumonia. The carcinoma of the liver may be accepted without question, but one might with some justification question the type of the lipoid storage disease. In favor of the correctness of the diagnosis are the typical histologic appearance and distribution of the lipoid-containing cells and the demonstration of the lipoid material by some of the accepted staining methods. Against the diagnosis are the absence of marked enlargement of the spleen, the absence of (or failure to note) the yellow coloration of the spleen said to be typical of the condition and the partial failure of some of the staining methods to produce positive results. The spleen was slightly enlarged, and the yellow coloration might have been absent because the condition had not progressed to its usual termination. The relatively slight involvement of the liver supports the idea that the disease was not fully developed. The failure of the staining methods may be explained by the length of time during which the liver was in Kaiserling's solution 1 and the splenic tissue in waste alcohol. The distribution of the lipoid deposits and the lack of a wavy fibrillar appearance of the cytoplasm of the foam cells with Mallory's aniline blue stain rule out Gaucher's disease. The distribution of the lipoid substance does not so clearly rule out Hand-Schüller-Christian disease, and neither the brain nor the skeletal system was examined, but the absence of a tendency to granulation and cicatrization about the lipoid deposits is in favor of Niemann-Pick disease and against Hand-Schüller-Christian disease. Pick [4b] stated, however, that the latter disease occurs predominantly in the male sex, whereas Niemann-Pick disease occurs most often in females. Both are familial diseases, with a predilection for the Jewish race. This infant was of German parentage, and there was no history of amaurotic familial idiocy, a condition not infrequently associated with Niemann-Pick disease. The facts that persons with Niemann-Pick disease almost invariably die before the age of 2 years and that the symptoms of Hand-Schüller-Christian disease rarely appear before the second year

do not help in making the differential diagnosis here, because this infant's illness followed the typical course and termination of primary carcinoma of the liver, and I do not believe that the disturbance in lipoid storage produced death.

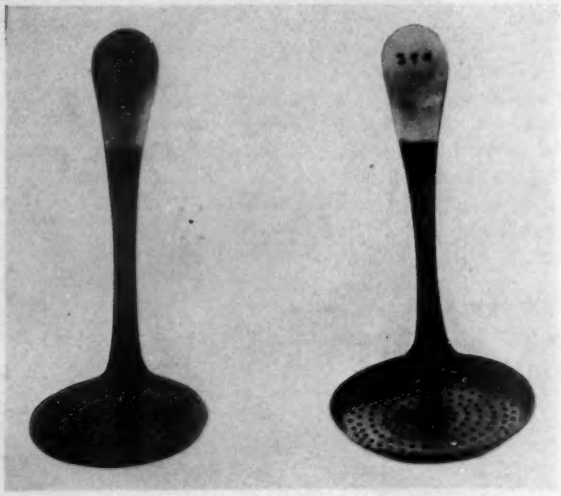
There is no evidence to suggest any causal association of these two rare diseases in the same person. While the point of view is not satisfying, the association must be attributed to coincidence. The absence of jaundice and ascites is explainable by the absence of both extrahepatic and intrahepatic biliary and portal obstruction. The rapid growth of the tumor, the differentiated type of cell, the absence of jaundice, ascites and cirrhosis of the liver, the situation and degree of metastasis and the rapidly fatal termination, with extreme emaciation, are all typical of the usual carcinoma of the liver of infancy.

# Laboratory Methods and Technical Notes

## PERFORATED SPOONS FOR HANDLING TISSUE SECTIONS (FROZEN, PARAFFIN AND PYROXYLIN)

GEORGE W. LUBINSKY, A.B., NEW YORK

The use of glass hooks for handling tissue sections becomes increasingly difficult as the sections become larger. This is especially true of frozen sections but applies as well to paraffin and pyroxylin sections,



Photograph of a set of spoons, made of brass and gold plated.

particularly to large pyroxylin sections. Frayed and incomplete sections are the result with frozen material, and tedious, long-drawn-out procedures are the rule with embedded material.

This difficulty was encountered in this laboratory in handling frozen whole brain sections. To facilitate this work, I devised a pair of perforated spoons.

### DESCRIPTION OF SPOONS AND THEIR USE

The two spoons are a lower one, with a narrow upturned rim and a flat bottom in which are multiple perforations, and an upper one which fits snugly into the first and is like it in all respects except that the rim is not upturned (figure).

From the Neuropathological Division of the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Department of Neuropathology, Neurological Institute of New York.

After a section is cut, it is spread on the lower spoon in fluid. When lifted out of the fluid, the section remains evenly spread on the spoon. The upper spoon is pressed down firmly on the section and the lower spoon as the section is dipped into any subsequent fluid (water, stain or dehydrating or clarifyng agent). The tissue is thus kept smoothly spread. Once the section is in a given fluid, the tissue may be gently agitated in the fluid if necessary. The perforations in the spoons permit free access of the fluid to the tissue and also cause it to flatten out smoothly as the fluid escapes through the perforations when the spoon is lifted. In addition to permitting this easy handling of the section, the spoon device obviates the necessity of fishing about in opaque staining solutions for tissues.

A description of the use of the spoons in handling large frozen brain sections (150 mm. in diameter) may serve as a model procedure. Frozen sections from 50 to 150 microns in thickness are cut from a slab of brain 1 cm. thick, serially sectioned in the frontal plane.

1. The section is transferred to the lower spoon from the microtome knife with the aid of a camel's hair brush.

2. The spoon is immersed gently in water.

3. The floating tissue is spread with a glass rod and camel's hair brush.

4. The spoon is raised slowly, one side slightly higher than the other. The tissue is thus received on the perforated surface of the spoon without wrinkling. It is raised and drained of fluid, which escapes through the perforations.

5. Before it is immersed in the next fluid, the section is covered with the upper spoon, which is firmly pressed against the lower. The spoons and tissue are then slowly lowered into the fluid, one edge first. The upper spoon may then be removed, or the staining may be carried on with the tissue between the spoons.

These spoons may be made of brass and gold plated, of glass or of black and white hard rubber. Hard rubber was tested by continuous immersion for two weeks in 2 per cent chromic acid, 50 per cent lithium carbonate, 2 per cent silver nitrate, 5 per cent nitric acid, 5 per cent hydrochloric acid, 5 per cent ferric ammonium sulfate, 50 per cent glacial acetic acid, xylene, solution of formaldehyde and absolute alcohol. At the end of this time no erosion, discoloration or increased brittleness resulted.

# Notes and News

**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Howard J. Shaughnessy, for the past year associate professor of bacteriology and public health at the University of Colorado, is again director of laboratories of the state department of health of Illinois, a position he held from 1930 to 1937, when he went to Denver.

Thomas B. Davie, professor of pathology in the University of Bristol, has been appointed to the George Holt chair of pathology, University of Liverpool, in succession to J. H. Dible, now professor of pathology in the Postgraduate Medical School, London.

The fiftieth anniversary of the Institut Pasteur in Paris will be celebrated on October 26, at the opening session of the first international congress of French-speaking microbiologists.

The University of California will establish a laboratory for the study of sylvatic plague under the auspices of a sylvatic plague committee. The Rosenberg Foundation of San Francisco has granted $24,000 for this purpose.

The Squibb Institute for Medical Research, a new institute in process of organization by E. R. Squibb & Sons at New Brunswick, N. J., will occupy a $750,000 laboratory. The director will be George A. Harrop. The four main divisions are experimental medicine, pharmacology, bacteriology, including virus diseases, and organic chemistry.

**Society News.**—A. T. Henrici has been elected president and I. L. Baldwin secretary-treasurer of the American Society of Bacteriologists.

**Awards.**—The Eli Lilly & Co. annual prize for outstanding contributions to bacteriology and immunology by an American investigator under 31 years of age has been awarded by the American Society of Bacteriologists to Jerome T. Syverton, University of Rochester, for his work on filterable viruses.

**Medicolegal Conference.**—The Massachusetts Medicolegal Society will hold a conference on various medicolegal topics on Oct. 4 and 5, 1938. There will be demonstrations and discussions of medicolegal methods and lectures on legal procedures, expert testimony and other pertinent topics. The arrangements are in the charge of Dr. William H. Watters, 270 Commonwealth Avenue, Boston. Physicians and lawyers interested in medicolegal work are invited to attend. There will be no registration fee.

# Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

## Experimental Pathology and Pathologic Physiology

Changes in the Teeth Following Parathyroidectomy: Effects of Different Periods of Survival, Fasting and Repeated Pregnancies and Lactations on the Incisor of the Rat. I. Schour, S. B. Chandler and W. R. Tweedy, Am. J. Path. **13**:945, 1937.

The incisor of the parathyroidectomized rat shows disturbances in calcification and in development. The essential character of the reaction varies with the length of the period of survival. In short periods up to twenty days the postoperative dentin indicates denser calcification than normal and is demarcated from the preoperative dentin by a distinct ring, which stains deeply with hematoxylin. This ring, which is formed at the time of the operation and which is also found in other related experimental conditions, appears to be an acute response to a shock to calcium metabolism and may be referred to as the calciotraumatic ring. The denser calcification of the postoperative dentin may be a result of the retention of calcium that is reported to persist during the early part of the period of survival. In longer periods of survival the changes consist in defective calcification and defective formation of enamel and dentin. The severity of the changes increases with increase in the time of survival. The most characteristic change consists in an aberration in the calcification of the dentin. The dentin shows an irregular and accentuated alternation of zones of different densities in calcification. This severe fluctuation, rather than lack of calcification, is responsible for the readiness of the parathyroprival dentin to fracture. The histologic changes are aggravated by fasting on every seventh day. The most severe alterations were found in animals that were subjected to repeated pregnancies and lactations. Histologic examination shows no evidence of a withdrawal of calcium from the calcified tissues of the teeth. The findings differ from those reported in the literature in the following respects: dense calcification of dentin during the early part of the period of survival; less severe changes for the same period of survival, and a less prominent rachitic-like width of predentin. These differences may be ascribed chiefly to a difference in diet. (See also "The Effect of Parathyroid Extract and Calciferol on the Incisor of the Rat " by I. Schour and others [*Am. J. Path.* **13**:971, 1937]).

From Authors' Summary.

Experimental Hypertension. H. Goldblatt and W. B. Wartman, J. Exper. Med. **66**:527, 1937.

Section of the anterior spinal nerve roots from the sixth thoracic to the second lumbar inclusive did not prevent or significantly and permanently reduce hypertension produced by constricting the main renal arteries of dogs. The significance of these results for the pathogenesis of human and experimental hypertension is discussed.

From Authors' Summary.

Action of Sulfanilamide in Experimental Streptococcic Empyema. F. P. Gay and A. R. Clark, J. Exper. Med. **66**:535, 1937.

Sulfanilamide prevents the evolution of an invariably fatal streptococcic empyema in rabbits when it is given repeatedly and in sufficient doses subcutaneously. Complete sterilization of the inoculated cavity occurs on approximately

the second day. The serum, defibrinated blood and artificial pleural exudate of similarly treated animals inhibit the growth of the same streptococcus in the test tube, but even repeated doses of such treated blood serum fail to sterilize the culture. The coccal chains grown in such drugged serum are elongated and present pleomorphic and metachromatic organisms and may give rise to colonies that are at first less predominantly mucoid in appearance. Such organisms have, however, lost little, if any, of their virulence. Cooperation on the part of locally derived clasmatocytes is apparently required in complete sterilization of the animal body. This conclusion is reached not only by a process of exclusion from comparison with the test tube results but through the direct histologic demonstration of a precocious and increasing mobilization of clasmatocytes in the parietal and visceral pleura of treated animals. In other words, sulfanilamide apparently produces a bacteriostasis sufficiently marked to protect the accumulated clasmatocytes. There is direct evidence that the drug does not in itself stimulate the mobilization of the macrophages. There is no evidence that the cell reaction which finally accounts for disposal of the organisms is other than local.

FROM AUTHORS' SUMMARY.

THE ROLE OF THE SPLEEN IN EXPERIMENTAL POLIOMYELITIS. E. H. LENNETTE, J. Exper. Med. **66**:549, 1937.

No difference in susceptibility to infection between splenectomized and nonsplenectomized monkeys was observed when virus was injected intravenously in large amounts. In a small series of animals, the results suggested that splenectomy decreased resistance to amounts of virus subinfective for nonsplenectomized monkeys. Splenectomy subsequent to intravenous or intrasplenic inoculation of amounts of virus subinfective for nonsplenectomized monkeys did not render the animals susceptible. Following intravascular injection, the virus accumulates in the spleen, where it can be detected twenty-four hours after injection but not at three or at five days. Evidence that splenectomy interferes with the formation of antibodies is presented and discussed. Lennette was unable to demonstrate virus in crude extracts of spleens of poliomyelitic animals but was able to do so in extracts of perfused spleens. This may be due to removal of a neutralizing factor. Attempts to immunize test animals with the virus present in poliomyelitic spleens were negative.

FROM AUTHOR'S SUMMARY.

PANMYELOPHTHISIS WITH HEMORRHAGIC MANIFESTATIONS IN RATS ON A NUTRITIONAL BASIS. P. GYÖRGY and others, J. Exper. Med. **66**:579, 1937.

During the twelve months ending with May 1937, György and his co-workers observed 72 rats that manifested typical symptoms of panmyelophthisis. The disease may start as agranulocytosis, thrombocytopenia or pure red cell anemia, leading progressively, often rapidly, to aleukia haemorrhagica with its typical manifestations (epistaxis, melena, hematuria, purpura). The white cell, red cell and platelet counts were correspondingly low with pronounced granulocytopenia (from 0 to 4 per cent). Bone marrow smears and histologic observations were consistent with the diagnosis of panmyelophthisis. A frequent postmortem observation was adrenal hemorrhage. The pathogenesis of this experimental panmyelophthisis and hemorrhagic diathesis is confined to special nutritional conditions—these diseases were observed in rats kept on a diet deficient in vitamin $B_6$, containing cane sugar and supplemented with vitamin $B_1$ and crystalline natural or synthetic lactoflavine. Lack of vitamin $B_6$, however, is not a necessary condition, since the disease was encountered in the majority of the animals after the specific deficiency disease which became apparent in rats kept on the $B_6$-free diet was successfully treated with purified $B_6$ preparations. Even in the untreated animals kept on the $B_6$-deficient diet acrodynia was, as a rule, in distinct remission before symptoms of panmyelophthisis and hemorrhagic diathesis became manifest. By adding Peters' eluate (Kinnersley, H. W., and others: *Biochem. J.* **27**:225, 1933) to the basal diet, panmyelophthisis could be prevented and in animals in which it was recognized

in an early stage, cured. In view of these facts it is suggested that Peters' eluate contains a specific maturation factor which, being different from lactoflavine, vitamin $B_6$ and probably also from the so-called filtrate factor, constitutes another distinct component of the vitamin $B_2$ group. Bacteriologic studies brought forward no conclusive evidence for an infectious agent in the experimental panmyelophthisis in these rats. The possible relation of this new disease in rats to aleukia haemorrhagica and its partial manifestations in man, as well as to the so-called Waterhouse-Friderichsen syndrome, is discussed. Administration of aminopyrine, at least under the conditions chosen, failed to provoke panmyelophthisis in rats kept on the same diet as that given to rats in which the disease developed spontaneously.

FROM AUTHORS' SUMMARY.

CHANGES IN BLOOD VESSELS (CAPILLARY FRAGILITY) WITH INFLAMMATION. E. ZANDER, J. Exper. Med. **66**:637, 1937.

Suction with a partial vacuum of —70 mm. of mercury applied to normal skin of rabbits causes intracutaneous hemorrhage after an average time of eight minutes. Inflammation produced by various agents, including turpentine-killed streptococci, staphylococci, pneumococci or meningococci and filtrates from cultures of meningococci or typhoid bacilli, produces a series of changes that are almost uniform. Immediately after injection of the irritant there is a great increase in the resistance to the production of hemorrhage by suction, so that the time required may be from one-half hour to almost two hours. This increase in resistance is doubtless caused by the contraction of blood vessels following injection of the irritant. The period of increased resistance is soon followed by one of diminished resistance of the vascular walls, so that from twelve to twenty-four hours after injection hemorrhage occurs within from one to four minutes after suction. The subsequent course of events varies; resistance of the vascular wall to suction, designated for convenience capillary fragility, may return to normal after from two to nine days (observed with turpentine and with streptococci), or for several days it may considerably exceed this level (observed with staphylococci and with pneumococci), or it may remain at a low level for a week or more (observed with tuberculin). With inflammation in a sensitized animal (allergic inflammation) the preliminary period of resistance may be diminished and the appearance of capillary fragility hastened, so that hemorrhage occurs after two minutes of suction applied six hours after injection, and later it may fall to an even lower level (observed with hemolytic streptococci in sensitized animals). The preparatory injection of toxic substances, such as a meningococcus or a typhoid filtrate, used in the production of the Shwartzman phenomenon, causes inflammation with injury of small blood vessels indicated by susceptibility to hemorrhage with suction. It is probable that subsequent intravenous injection causes hemorrhage by further injury to these injured blood vessels.

FROM AUTHOR'S SUMMARY.

## Pathologic Anatomy

ENCEPHALOMYELITIS COMPLICATING MEASLES. N. MALAMUD, Arch. Neurol. & Psychiat. **38**:1025, 1937.

In 2 cases of cerebral complications following measles, Malamud found scattered foci of pathologic change in the central nervous system. In a boy aged 6, who died three days after the appearance of the rash and twelve and one-half hours after the onset of severe symptoms pointing to involvement of the central nervous system, the foci were around small veins, which were infiltrated with lymphocytes and plasma cells, and consisted of "a cellular syncytium." The cells were "almost exclusively polyblastic glia cells," with which the author includes "microglia cells, large gitter cells with small dark nuclei and irregular cell bodies, and small mononuclear forms (Wickman's polyblasts)." In addition, there were also present small perivascular areas of demyelinization. In the second

case, that of a boy aged 8, who died three weeks after the onset of measles, the foci were also perivenous but consisted of demyelinated nerve fibers. Perivascular hematogenous infiltrations were practically absent in this case, but gitter cells loaded with "prelipoids" were here very numerous and in the cortex were mixed with proliferated microglia and macroglia. Malamud concluded from his observations that measles may cause encephalomyelitis, the disseminated foci of which are not identical with those of multiple sclerosis, and that the pathologic features may vary, depending on the age of the changes. In the first case the foci were of three days' duration and were distinctly inflammatory; in the second case they were of three weeks' duration and exhibited degenerative phenomena.

GEORGE B. HASSIN.

LESIONS OF THE RECTUS ABDOMINIS MUSCLE. T. S. CULLEN and M. BRÖDEL, Bull. Johns Hopkins Hosp. **61**:295 and 317, 1937.

Hemorrhage into or beneath the rectus abdominis muscle is rather rare. It may take place in any part of the muscle but is usually in the part below the umbilicus. In some cases the muscle is torn; in others, branches of the deep epigastric artery or vein have ruptured; in still other cases there have been both rupture of the muscle and tearing of one or more blood vessels. As noted in Max Brödel's illustration, the posterior sheath of the rectus is missing in its lower portion; consequently, when the bleeding is below the navel, the blood usually lies between the muscle and the peritoneum. This causes peritoneal irritation and produces symptoms simulating in nearly every particular those of an acute abdominal lesion. In those cases in which the sheath of the rectus has ruptured anteriorly, blood escapes into the fat and there may be an ecchymosis of the skin. In such cases a correct diagnosis may be made more readily. Sometimes rupture of the rectus muscle has been caused by direct violence; usually, however, the patient has for some time been suffering from some debilitating disease, and slight exertion has been followed by sudden abdominal pain with the development of the hematoma. In typhoid and influenza there has often been extensive waxy degeneration of the rectus muscle — Zenker's degeneration. In such cases the muscle is brittle and tears easily. This degeneration has undoubtedly been the causative factor in rupture of the muscle. In some patients with disease of the gallbladder, in some with cardiac lesions and in some suffering from bronchitis, rupture has occurred. Hematoma of the rectus has occasionally been noted during pregnancy.

FROM AUTHORS' SUMMARY.

EFFECT OF THROMBOPHLEBITIS ON THE VENOUS VALVE. E. A. EDWARDS and J. E. EDWARDS, Surg., Gynec. & Obst. **65**:310, 1937.

Phlebitis, with organization and recanalization of its attendant thrombosis, has a profound effect on the valves of the involved vein. Complete thrombosis produces, actually or functionally, a valveless vein.

If the cusp is closed and projecting into the lumen at the time of the formation of the thrombus, it lies, a passive structure, fragmented by hemorrhage and inflammatory exudate, in the center of the organizing thrombus, becomes adherent to the wall of the vein at the sinus and to its own folds and its fellow cusp and is traversed by capillaries and fibroblasts. As organization proceeds, the capillaries widen and coalesce, constantly increasing the fragmentation of the cusp, and by the time the capillaries have become sizable the cusp ceases to exist save in the form of fragments of elastic tissue lying between the lumens. Finally, after a single lumen has been produced, only traces of elastic tissue can be found in the irregularly thickened intima, to mark the site of the valve.

Should the cusp be near the wall of the vein at the moment of thrombosis, the earliest organization binds it to the intima, so that the later changes of organization and recanalization disrupt it less than in the former case but leave the vein functionally just as valveless, because the cusp cannot project into the lumen. In

the event of incomplete, or mural, thrombosis there may occur changes in the cusps that are comparable to stenosis and insufficiency of cardiac valves. While fragmentation is minimal and usually limited to the base, there takes place nevertheless an addition of new connective tissue which binds the proximal part of the cusp to the wall of the vein, with thickening and shortening of varying degree. Stenosis may also be occasioned by organization of a thrombus in the valve sinus, producing a pad of tissue that prevents wide opening of the cusp. As in complete thrombosis, the cusp may be rendered functionless by adhesion of its entire length to the wall of the vein. A special form of adhesion with resultant insufficiency is seen in the valve guarding a tributary to a vein undergoing thrombosis. In this situation one or both cusps may be caught in the thrombus of the main trunk, either by the free edge or by the whole length. Organization of the thrombus will destroy these cusps or pull them far apart and maintain them in a constant open position. Such a condition is exemplified in the valves of the perforating veins of the leg when a thrombus involves the deep veins. In the event of insufficient recanalization the collateral veins dilate to take over the blood flow and produce a necessary insufficiency of the valves of these collaterals. This condition is observed in the veins of the trunk after occlusion of either vena cava. The opinion is expressed that the studies explain at least one of the reasons for the poor venous circulation following phlebitis.

FROM AUTHORS' SUMMARY. (WARREN C. HUNTER.)

TRAUMATIC AUTOPLASTIC TRANSPLANTATION OF SPLENIC TISSUE. A. F. B. SHAW and A. SHAFI, J. Path. & Bact. **45**:215, 1937.

A case is described of splenectomy for traumatic rupture of the spleen in which a number *of* nodules were found in the peritoneum and elsewhere which are believed to have been autoplastic transplants derived from grafts of splenic tissue. The pulp, which actively arrested tubercle bacilli, is the only component of the transplants with the structure and functions of spleen. The emergency splenic function of contraction must have been lacking, owing to the absence of musculo-elastic capsule and trabeculae. The capsule is provided by the host's tissues only as a supporting and nutrient stroma. It is doubtful if transplants grow purely as an attempt to compensate for the spleen. Transplants probably develop only after severe trauma of the spleen, and evidence is advanced to show that removal of a diseased spleen, even though traumatized at operation, is rarely, if ever, followed by successful transplantation. A review of the literature on this rare condition shows that the growths have been regarded either as true accessory spleens or as derived from spleen-forming rests in the peritoneum. In only a single instance (Beneke) were they accepted as transplants. In 5 cases of severe bilharziasis in which an enlarged spleen had been removed from one to twenty-two years before death no constant or characteristic changes were found which could be interpreted as compensating for the spleen. Accessory spleens are probably formed at the same time as the main organ. Their capacity to hypertrophy is restricted, as they were found to differ little according to whether the spleen was normal or enlarged or had been removed. FROM AUTHORS' SUMMARY.

ARSPHENAMINE ENCEPHALITIS. D. S. RUSSELL, J. Path. & Bact. **45**:357, 1937.

Three examples of so-called hemorrhagic encephalitis following medication with arsphenamine are described. Stress is laid on the occurrence of nonhemorrhagic perivascular areas of necrosis and demyelinization in addition to hemorrhages. The possibility is discussed that both lesions are due to sensitization, the nonhemorrhagic variety representing a less intense reaction on the part of the tissues. The resemblance of the nonhemorrhagic lesions to those reported in cases of encephalomyelitis complicating antirabic treatment and in cases of postvaccinial encephalomyelitis is noted. FROM AUTHOR'S SUMMARY.

WHITE PULMONARY INFARCTS. F. NIENDORF, Beitr. z. path. Anat. u. z. allg. Path. **100**:149, 1937.

Although anemic pulmonary infarction is so rare that its occurrence is denied by experienced pathologists, Niendorf, reviews the cases reported in the literature and describes 5 of his own.

The conditions essential for its development are a preceding croupous or confluent fibrinous bronchopneumonia accompanied by a purely mechanical, noninfected embolic or thrombotic arterial obstruction. Bacteria are probably not responsible in that their presence would lead not to infarction but to abscess or gangrene.

The infarcted areas are subpleural in location, firm, distinctly pyramidal in form, with the base directed toward the pleura, pale gray to yellowish and surrounded by inflammation. The pallor of the infarct is due to the absence of a collateral circulation and to the fibrinous and cellular exudate, which mechanically prevents the entrance of the red blood cells into the alveolar spaces. Microscopically, the walls of the alveoli are necrosed, and the alveolar exudate is striking by reason of the complete or almost complete absence of red blood cells.

For the diagnosis of white infarcts the following requirements must be satisfied: (1) necrosis; (2) absence of a collateral circulation and of red blood cells in the alveolar exudate; (3) arterial embolism or thrombosis; (4) an approximate pyramidal shape; (5) organization and fibrosis with clinical recovery as is the case with simple anemic infarcts of the kidney and spleen. R. J. LEBOWICH.

LARYNGEAL CYSTS. R. HÜCKEL, Zentralbl. f. allg. Path. u. path. Anat. **68**:113, 1937.

Laryngeal cysts, although of infrequent occurrence, have been studied sufficiently to allow the following grouping: congenital, retentive, traumatic and lymphatic. The cyst reported here was of the lymphatic group and was found incidentally in postmortem examination of the body of a man 56 years old who had died of hypostatic pneumonia. The pea-sized mass was smooth, broadly based and sessile and occurred in the middle of the left aryepiglottic fold. In serial sections it was lined with stratified noncornified epithelium, which was nowhere in contact with the mucous membrane of the larynx. The cyst was surrounded by a small rim of lymphocytes devoid of germinal centers. The content was desquamated epithelium and leukocytes. GEORGE J. RUKSTINAT.

PERIVASCULAR SCARS IN THE MYOCARDIUM. E. NORINDER, Acta path. et microbiol. Scandinav. **14**:305, 1937.

Examination of the heart muscle in 324 cases revealed that perivascular fibrosis is common. Such scars are common in rheumatic hearts but cannot be regarded as specific for rheumatism. Perivascular myocardial fibrosis does not have a common genesis with rheumatic lesions but must be regarded as a result of an "allergic-hyperergic" reaction to various toxic products.

## Microbiology and Parasitology

TYPHOID CARRIERS: A STUDY OF ENVIRONMENTAL AND OTHER FACTORS BEARING ON PERIODICITY. R. MCBURNEY, J. Infect. Dis. **61**:122, 1937.

Weekly examinations of the stools of 7 typhoid carriers, covering fifty-two weeks, showed that the incidence of the carrier state was 15 per cent higher among females than among males. There was no correlation among adults referable to age, duration of the carrier state and percentage of positive cultures.

The well established carrier group gave positive cultures 87 per cent of the time. There was practically no negative phase among them.

Temperature, humidity, diet, mode of living, minor illness and other factors studied did not alter the carrier state. Increasing temperatures and humidity during the warmer months did not produce a positive phase among the 4 periodic carriers whose cultures were consistently negative.

Among the 7 well established carriers there were never more than two negative cultures in any one week, and no such carrier gave negative cultures more than two successive times. In 24 weekly fecal specimens from the 7 well established typhoid carriers, typhoid bacilli averaged 49.45 per cent of the bacterial flora.

In considering the results obtained from the whole group, studied throughout the year, coupled with the data from the hot room experiments, it seems justifiable to conclude that changes in temperature, humidity, environmental and other factors studied had no effect on periodicity as far as this particular group is concerned.

FROM AUTHOR'S SUMMARY.

SPIROCHETOSIS IN WHITE MICE PRODUCED BY THE INOCULATION OF MATERIAL FROM CHRONIC PULMONARY ABSCESS, ULCERATIVE STOMATITIS AND PYORRHEA ALVEOLARIS. C. WEISS, J. Infect. Dis. **61**:143, 1937.

Material containing various types of spirochetes, staphylococci, aerobic and anaerobic streptococci, Bacterium melaninogenicum, fusobacteria, organisms of the Bacteroides group, etc., was obtained from areas of pyorrhea and abscesses of the gum, from ulcerative lesions of the mouth and from the lung of a patient with chronic pulmonary abscess. By inoculation of this material intraperitoneally, subcutaneously or intracutaneously, directly or after preliminary passage in series from one guinea pig to another, experimental spirochetosis was induced in white mice. These animals usually killed off the contaminating bacteria, permitting the spirochetes to develop alone in the blood stream. The initial time of appearance of the parasites varied from the twenty-eighth to the seventieth day, but by repeated passage of infected blood from mouse to mouse it was possible to shorten the time markedly and to increase the number of spirochetes in the blood stream. A similar effect was accomplished by injecting the bacteria into mice which had been splenectomized and in which the reticuloendothelial cells had been partially blocked by repeated injections of india ink. At necropsy the spirochetes were demonstrable either by means of the dark field or in sections stained by Dieterle's method, in several of the organs, including the ovary, testis, lung, liver and kidney. The spiral organisms were confined to the inside of the small blood vessels or capillaries, and there was no evidence of inflammatory reaction in any of the organs.

FROM AUTHOR'S SUMMARY.

INFLUENCE OF GLYCEROL ON SKIN NECROSIS BY STAPHYLOCOCCUS TOXIN. M. L. SMITH, Brit. J. Exper. Path. **18**:265, 1937.

Glycerol inhibits necrosis produced in the skin of guinea pigs by staphylococcus toxin. The low values sometimes obtained when staphylococcus antitoxin is titrated by the intracutaneous method are attributable to the use of a standard serum diluted in glycerol. The inhibiting action of glycerol is specific for the toxin of the staphylococcus. Inhibition is also produced by ethylene glycol, sucrose and dextrose. The action is one of partial destruction of the toxin.

FROM AUTHOR'S SUMMARY.

CENTRIFUGATION STUDIES. F. F. TANG, W. J. ELFORD and I. A. GALLOWAY, Brit. J. Exper. Path. **18**:269, 1937.

The rates of sedimentation of a bacteriophage for Megatherium and of the viruses of equine encephalomyelitis and louping ill when under the influence of centrifugal forces have been studied by the inverted capillary tube technic. The values for particle size indicated for the respective active agents are in general agreement with the earlier values obtained by ultrafiltration analysis.

FROM AUTHORS' SUMMARY.

MORPHOLOGIC STUDIES ON VACCINIA VIRUS CULTIVATED IN THE DEVELOPING EGG. F. F. TANG and H. WEI, J. Path. & Bact. **45**:317, 1937.

Examination of the virus of vaccinias cultivated in the chorioallantoic membranes of chick embryos revealed two types of cytoplasmic inclusions, large and small. The large form appears early in development and may be regarded as a sort of colony in which the small inclusions lodge. Many cells contain only the small bodies, which may appear as diplococcoids, short chains or bacillary structures. In all probability the large form corresponds to the classic Guarnieri body and the small to the Paschen body. These structures have been seen in the living membrane by transmitted light, with or without a supravital stain, by dark field illumination and in stained smears. Dark field examination was not satisfactory on account of the thickness of the membrane. Two possible developmental cycles of the virus are suggested, and the changes in virulence during egg propagation of the virus are discussed.

FROM AUTHORS' SUMMARY.

CULTIVATION OF SMALLPOX VIRUS ON THE CHORIOALLANTOIC MEMBRANE OF THE HEN'S EGG AND THE PREPARATION AND PRACTICAL USE OF SUCH CULTURES. I. PERAGALLO, Zentralbl. f. Bakt. (Abt. 1) **138**:310, 1937.

Peragallo cultivated the virus of vaccinia in tissue culture, using both the method of Carrel and Rivers and that of Goodpasture and Woodruff. He found that the latter method was particularly useful and that the virus could be maintained serially with complete sterility. The virus seemed to be identical with that of cowpox in that Guarnieri bodies were demonstrable in the chorioallantoic membrane and in typical pustules formed in the skin of rabbits after injection of the culture virus. Culture virus conferred immunity to cowpox virus when injected into human beings, and the serums from these manifested neutralizing properties as long as two years after injection. Peragallo concludes that the cultivation of cowpox on the chorioallantoic membrane of the hen's egg is a practical method for the preparation of such material free from the dangers of bacterial infection and that the culture virus gives a solid immunity against cowpox in animals and in man.

PAUL R. CANNON.

PATHOGENESIS OF CROUPOUS PNEUMONIA. H. GROSSMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:144, 1937.

Rabbits were inoculated intracutaneously with living pneumococci, types II or VI, with resulting marked local reactions. From three to four weeks later living cultures of the homologous strains were instilled intranasally. In these rabbits pneumococcic lesions developed which in most of them resembled histologically lobar pneumonia of man. In the controls the lesions consisted mainly of hemorrhages, necrosis and atelectasis. Septicemia was much more common in the controls than in the animals previously given injections. Grossmann concludes that the results favor the opinion that lobar pneumonia is an allergic reaction after previous sensitization.

I. DAVIDSOHN.

## Immunology

EFFECT OF ACIDOSIS ON THE PRODUCTION OF IMMUNE BODIES IN THE RABBIT. W. W. BRANDES and A. B. CAIRNS, J. Immunol. **32**:137, 1937.

Rabbits rendered acidotic by intravenous injections of ammonium chloride responded poorly in the formation of agglutinins and precipitins for typhoid bacilli and ovalbumin whether these were inoculated separately or simultaneously. The effect on the production of both antibodies was similar.

I. DAVIDSOHN.

GROUP-SPECIFIC DIFFERENTIATION OF HUMAN FECES WITH SPECIAL REGARD TO AB GROUP. I. MOHARRAM, J. Immunol. **32**:229, 1937.

Normal stools contain group-specific agglutinogens. Aqueous extracts reacted with the corresponding isoagglutinins, and alcoholic extracts reacted with the rabbit antisheep hemolysin, produced by injections of human red cells of type A. The stools of persons of types $A_1B$ and $A_2B$ showed the group substances in "eliminators" as well as in "noneliminators" of the group substances. The extracts of stools of the $A_2$ and $A_2B$ groups reacted somewhat more feebly than did the extracts of the $A_1$ and $A_1B$ groups with the corresponding antibodies.

I. DAVIDSOHN.

THE LIVER IN ACUTE ANAPHYLACTIC SHOCK IN THE GUINEA PIG. R. H. BROH-KAHN and I. A. MIRSKY, J. Immunol. **32**:409, 1937.

Anaphylactic shock was not prevented in sensitized guinea pigs by removing the liver and spleen according to the technic of the authors. This indicates the irrelevance of the reticuloendothelial symptoms as represented in the liver and spleen for the mechanism of the anaphylactic shock.

I. DAVIDSOHN.

RESPONSE OF INFANTS TO TYPE I PNEUMOCOCCUS CARBOHYDRATE. J. A. V. DAVIES, J. Immunol. **33**:1, 1937.

In contrast to adults, who readily produce circulating type-specific pneumococcus antibodies following injections of type-specific pneumococcus carbohydrates, only 1 of 15 infants from 2 to 15 months of age produced bactericidins, opsonins, agglutinins and protective antibodies in response to injections of the so-called type I cellular ("CC") carbohydrate of Wadsworth and Brown.

I. DAVIDSOHN.

INFLUENCE OF URETHANE ON ANAPHYLACTIC REACTIONS. L. FARMER, J. Immunol. **33**:9, 1937.

Strips of uterus from virgin guinea pigs sensitized to horse serum did not contract on addition of the antigen to the Ringer bath if ethyl carbamate (urethane) was added to the bath prior to introduction of the antigen. After the ethyl carbamate was washed out, further addition of antigen did not cause a contraction. This indicates that ethyl carbamate did not prevent the desensitizing action of the previously added antigen. The correctness of this interpretation was established by proper controls. The prevention of anaphylactic shock in sensitized guinea pigs by ethyl carbamate is explained by the latter's direct action on the bronchial musculature. The ability of this substance to prevent contraction (the effect of the liberation of "histamine-like" substances) without interference with the union of the antigen with the muscle tissue, as evidenced by the desensitizing effect, corroborates the two stage conception of anaphylaxis of Dale. Chloralose (a compound of chloral hydrate and dextrose) had the same effect as urethane.

I. DAVIDSOHN.

M AGGLUTINOGENS IN MONKEYS. K. LANDSTEINER and A. S. WIENER, J. Immunol. **33**:19, 1937.

Landsteiner and Levine in 1928 reported the finding of the property M in chimpanzees but not in other monkeys. The present reinvestigation with a number of agglutinating serums showed the presence of the M property in baboons, Macacus rhesus, the Java macaque, Sooty Mangabey, the green monkey and the white spider monkey. The serums showed marked differences; some agglutinated the red cells of all the examined animal species; others, those of only a few. The agglutinogen M appears to be limited to the blood of the primates. Absorption experiments demonstrated qualitative differences between the M factor of man

and that of some monkeys. There seems to be some correlation between the place in the zoologic scale and the degree of similarity between the M substances in the blood of monkeys and of man. In human blood, the M factor is an individual characteristic, while in monkeys it is a species characteristic. Rabbits immunized with rhesus blood yielded serums which equaled, after proper absorption, the most potent human anti-M serums.

I. DAVIDSOHN.

ANTIMENINGOCOCCIC VACCINATION. N. SILVERTHORNE, J. Immunol. **33**:51, 1937.

Silverthorne presents a modification of the technic for testing the bactericidal power of blood. Guinea pigs inoculated with a phenol-killed vaccine of meningococci resisted a subsequent inoculation of live meningococci. Their blood had no bactericidal properties before vaccination but had a marked titer of bactericidal activity after vaccination. Bactericidal power was also noted in the blood of the few control animals which did not succumb to the inoculation of live bacteria. Vaccination of 4 children from 10 months to 3 years of age who were without bactericidal properties in their blood was followed by the development of such properties. The action of the blood was specific against the type of meningococci used for vaccination.

I. DAVIDSOHN.

THE NATURE OF ANTIBODIES. S. B. HOOKER, J. Immunol. **33**:57, 1937.

This is the presidential address delivered by the author before the American Association of Immunologists. It presents his views on the nature of antibodies, with a critical analysis of the modern concepts based on colloidal chemistry.

I. DAVIDSOHN.

NORMAL BACTERIAL AGGLUTININS AND THEIR SIGNIFICANCE. E. O. JORDAN, J. Infect. Dis. **61**:79, 1937.

Cattle serum, horse serum and, in a few tests, monkey serum contain notable amounts of agglutinins for various species of bacteria. The serums of other mammals (sheep, man) contain smaller amounts, while those of rabbits and guinea pigs show little agglutinative power.

Different species of bacteria are agglutinated in varying degrees. Those species that are readily agglutinated by bovine serum are also readily agglutinated by equine serum. The three types of dysentery bacilli, Sonne, Flexner and Shiga, are agglutinated with decreasing intensity in the order named by both bovine and equine serums.

Agglutinins for the typhoid bacillus appear to be present in normal cattle serum in greater abundance than agglutinins for Salmonella aertrycke and for Salmonella enteritidis, organisms both of which might naturally be expected to infect cattle more frequently than the former.

"Normal" bacterial agglutinins do not appear to differ from the "immune" agglutinins produced by inoculation either in specificity as determined by the absorption test or in heat resistance.

A marked age difference exists in the amount of bacterial agglutinins: Adult cattle serum possesses agglutinative power for many species while the serum of calves is almost completely devoid of it. This is true not only for bacteria such as Brucella and Salmonella, which infect cattle naturally, but for the cholera vibrio and other organisms not usually forming a part of bovine environment.

FROM AUTHOR'S SUMMARY.

PNEUMOCOCCUS TOXIN AND ANTITOXIN. G. F. DICK and A. K. BOOR, J. Infect. Dis. **61**:228, 1937.

The production of a toxin or at least of a toxin-like substance in ordinary broth cultures of Pneumococcus is demonstrated. A method of concentrating this

substance is described. This purified and concentrated product makes practicable the production of an antitoxin and facilitates the further study of the toxin-antitoxin mechanism in pneumonia.

FROM AUTHORS' SUMMARY.

LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES. G. SHWARTZMAN, J. Infect. Dis. **61**:293, 1937.

Studies by Shwartzman and other investigators indicate clearly that in spite of superficial resemblance the factors provocative of the phenomenon of local skin reactivity bear no relationship to anaphylatoxins. There are some indications that the complexes of Forssman antigen and antibody may be endowed with provocative potency as regards the phenomenon. The provocative potency of whole blood of man and guinea pigs may be due to some in vivo interaction between the blood and the natural antibodies of the rabbit. The purpura-producing serums of Ledingham, Bedson and Roskam bear no relationship to the phenomenon of local skin reactivity.

FROM AUTHOR'S SUMMARY.

ARTIFICIAL OPSONIZATION OF BACTERIA. J. GORDON and F. C. THOMPSON, Brit. J. Exper. Path. **18**:390, 1937.

The previous work on tanning agents as artificial opsonins has been extended to include experiments with typhoid bacilli. The most constant and regular in their action as opsonins have been the iron and chromium salts. It has been noted that agglutination and opsonization are intimately related and that artificial opsonins always act as agglutinins but that substances which cause agglutination not necessarily act as opsonins. The vegetable tannins have been found to act as artificial opsonins with typhoid bacilli, but much more irregularly than with staphylococci. Because dyestuffs form nonhydrolyzable compounds with proteins, their action as artificial opsonins has been investigated. While no effects on typhoid bacilli, could be demonstrated, there was undoubted opsonization of some strains of staphylococci. A further interesting result was obtained in that protamine sulfate agglutinated and opsonized staphylococci.

FROM AUTHORS' SUMMARY.

EFFECT OF ASCORBIC ACID (VITAMIN C) ON TOXIN PRODUCTION BY CORYNEBACTERIUM DIPHTHERIAE. I. J. KLIGLER, L. LEIBOWITZ and M. BERMAN, J. Path. & Bact. **45**:415, 1937.

The addition of from 0.05 to 0.1 per cent cevitamic acid to culture medium suitable for the production of toxin by Corynebacterium diphtheriae renders filtrates of such cultures less toxic. The toxicity of vitamin-containing filtrates is only about one twentieth of that of control cultures. The cevitamic acid is rapidly oxidized in the cuture medium at a temperature of from 30 to 37 C., but in the presence of bacterial growth it is preserved longer than in sterile medium. Glutathione, another potent biologic oxidation-reduction agent, has no effect on the toxicity of cultures. Small amounts of cevitamic acid (from 0.1 to 0.2 per cent) mixed with toxin in a test tube and incubated at 30 C. render the toxin atoxic in from two to three days according to the concentration of the vitamin. This reaction occurs under both aerobic and anaerobic conditions. Contrary to the findings in growing cultures, the cevitamic acid disappears more rapidly in the toxin filtrate than in sterile medium. Toxin injected into guinea pigs leads to a rapid decrease in the vitamin C content of the adrenal glands. When a lethal dose of toxin is given, the vitamin C content may be reduced to nil. It is suggested that the cevitamic acid destroys the toxin by an oxidation-reduction reaction and that the action is similar to that produced by aldehydes in general.

FROM AUTHORS' SUMMARY.

BLOOD GROUPS OF AUSTRALIAN ABORIGINES. J. B. CLELAND and J. H. JOHNSON, J. Trop. Med. **39**:104, 1936.

In this investigation 725 pure blood natives of central Australia were examined, and 450 were found to belong to group A and 275 to group O. Of 149 pure blood natives of northwestern Australia, 101 belong to group A and 48 to group O. No natives were found belonging to group B or AB.

A. S. WIENER.

ABSORPTION OF BCG BY THE DIGESTIVE TRACT. S. R. ROSENTHAL, Ann. Inst. Pasteur **58**:652, 1937.

The administration of BCG by mouth to small unweaned guinea pigs produces a general but not diffuse reaction of the reticuloendothelial system. Among the animals inoculated with the organs of these guinea pigs some become sensitive to diluted tuberculin injected intradermally. The author found no modification of the cellular picture of the blood or of the peritoneal cavity attributable to a tissue response to BCG.

FROM AUTHOR'S SUMMARY.

VACCINATION AGAINST TYPHUS. J. LAIGRET, R. DURAND, J. BELFORT and J. LEFAUCHEUR, Arch. Inst. Pasteur de Tunis **26**:453, 1937.

In this report of 170 pages are presented the results of vaccinating 32,481 persons against exanthemic typhus with an "enrobed" living vaccine (a rat brain suspension "enrobed" or coated with egg yolk and oil), previously described (ibid. **25**:570, 1936). The usual result of the vaccination was an inapparent infection, although in 5 cases mild typhus followed the vaccination within from ten to sixteen days. These attacks occurred in Europeans, the vaccination of whom was regarded as entailing a light risk. Both vaccination and delousing thus far are recommended. Epidemiologic evidence indicates that definite immunity has been induced.

M. S. MARSHALL.

HETEROPHILIC ANTIGENS. G. BRUYNOGHE, Arch. internat. de méd expér. **12**: 397, 1937.

In this paper Bruynoghe deals with the multiplicity of heterophilic antigens. He discusses the complicated nature of the antisheep hemolysis which occurs normally in man and in several animal species. He noticed the inability of the guinea pig's kidney to absorb the antisheep agglutinins in the presence of infectious mononucleosis. He was apparently unaware that this observation had been made before by American investigators and that it led to the development of a test for differentiating between infectious mononucleosis and serum disease. Rabbits given injections of sheep red cells that were autoclaved at 120 C. produced an antisheep hemolysin that lysed somewhat human erythrocytes but without relation to any blood group. However, the same immune serum agglutinated selectively, in addition to sheep red cells, human red cells of type A but not those of type B. Similarly autoclaved human red cells of type A induced in rabbits the formation of specific anti-A agglutinins while autoclaved cells of type B did not stimulate the formation of anti-B agglutinins.

I. DAVIDSOHN.

BLOOD GROUPS IN TURKEY. E. BABAGAN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:1, 1937.

Among 2,000 Turkish students and soldiers, 34.15 per cent belonged to group O, 46.65 per cent to group A, 13.6 per cent to group B, and 6.6 per cent to group AB. The results differ from the previously reported data of Hirszfeld in the higher incidence of A and the lower incidence of B. A separation of those coming from the European part of Turkey (Istanbul) showed a lower number in group A (42.6 per cent) as compared with those in group A from Anatolia (49.82 per cent). Previous reports indicate similar differences in the incidence of A among the European and Asiatic Greeks.

I. DAVIDSOHN.

UNEQUAL DEVELOPMENT OF ISOAGGLUTININ TITERS IN IDENTICAL TRIPLETS. F. OTTENSOOSER and W. TOBLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:65, 1937.

What part does heredity and what part do external influences play in the formation of normal antibodies? Determinations of the titers of isoagglutinins in identical twins and triplets ought to throw light on this question. In identical triplets from 6 to 7 months old of group O, kept since birth under identical conditions, the titers of anitbodies for B differed considerably (2, 16, 8-16) while the titers of antibodies for A differed only very slightly (4, 2, 2-4). The results indicate that in identical siblings of group O only one of the isoagglutinins may show differences in titers and that external influences may exert an influence on the isoagglutinins.

I. DAVIDSOHN.

ANTIGENIC PROPERTIES OF FILTRATE OF A CULTURE OF STAPHYLOCOCCUS AUREUS. F. SCHAFF and P. ROBERT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **90**:192, 1937.

The filtrate was used in three forms: (1) unchanged; (2) detoxified by three weeks' incubation with 0.3 per cent formaldehyde; (3) detoxified by heating at 100 C. for two hours. Guinea pigs were sensitized with every one of the three products and after about seventy days the uterine horns of the animals were tested according to the Schultz-Dale technic. All products contained one common antigen from the culture medium. Four other antigenic substances differed in their sensitiveness to the heat and formaldehyde. One was resistant to heat and to formaldehyde, the second was thermolabile and formaldehyde resistant, the third was thermoresistant and formaldehyde labile and the fourth was thermoresistant and formaldehyde labile. Treatment with formaldehyde destroyed two of these four antigens, heat destroyed only one completely, while the other it changed to a hapten.

I. DAVIDSOHN.

THERMOSTABILE BACTERICIDAL SUBSTANCE IN SERUM OF FEVER PATIENTS. P. HJORTH, Acta path. et microbiol. Scandinav. **14**:412, 1937.

A bactericidal substance is found in human serum, especially during fever, artificial or otherwise, which is thermostabile and active against meningococci and certain other cocci. It is possible that this substance is complex in structure.

ALLERGY AND IMMUNITY IN EXPERIMENTAL TUBERCULOSIS. K. BIRKHAUG, Acta tuberc. Scandinav. **11**:25, 1937.

When virulent tubercle bacilli are injected in a minimal dose (0.000,01 mg., or approximately 500 bacilli) directly into the cervical lymph gland of the normal guinea pig and the internal organs are cultured at definite intervals dispersion of the bacilli is found to occur as early as three days after the injection, when the bacilli complete the circuit of the body through the lymphatic, venous and systemic circulation. In the guinea pig previously made excessively hypersensitive toward tuberculin through subcutaneous injection of virulent dysgonic tubercle bacilli, dispersion of virulent eugonic tubercle bacilli (approximately 500 bacilli) injected directly into the cervical lymph gland occurs as late as five weeks after the superinfection and the bacilli complete the circuit of the body in approximately from six to eight weeks. In the guinea pig rendered highly allergic through subcutaneous injection of virulent dysgonic tubercle bacilli and subsequently completely anergic (desensitized) by means of tuberculin, dispersion of virulent eugonic tubercle bacilli (approximately 500 bacilli) injected directly into the cervical gland occurs as late as seven weeks after the superinfection and the bacilli complete the circuit of the body in approximately from eight to eleven weeks. A comparative analysis of the macroscopic and microscopic observations on the tuberculous lesions reveals that these lesions are approximately 50 per cent more excessive, necrotic, caseous and extensive in the allergic than in the desensitized animal.

These experiments therefore support the contention that the allergic state is unnecessary for protection against tubercle bacilli and that the abolition of the hypersensitive state by means of tuberculin desensitization leaves immunity intact and greatly reduces the liability of incurring an extensive form of necrotic and caseous tuberculosis.

FROM AUTHOR'S SUMMARY.

## Tumors

THE REACTION OF STANDARD BREEDS OF RABBITS TO THE BROWN-PEARCE TUMOR. A. E. CASEY, Am. J. Cancer **31**:446, 1937.

Two hundred and seventy-six young adult male rabbits, representing twenty-one standard breeds, were given intratesticular injections of an emulsion of the Brown-Pearce tumor. A variation among the breed samples tested occurred in relation to the incidence and volume of the primary tumors, the incidence, the volume and the number of the metastases and the longevity and mortality after inoculation of tumor cells. This variation could not be explained on the basis of sex, site, age, physical condition, diet, housing, season or inoculum. It seemed to be due to variation in the inherent constitution of the pure breed strains used.

FROM AUTHOR'S SUMMARY.

PAPILLOMATOSIS IN THE FORESTOMACH OF THE RAT AND ITS BEARING ON THE WORK OF FIBIGER. W. CRAMER, Am. J. Cancer **31**:537, 1937.

In rats kept on a diet of bread and water and infected with Gongylonema neoplasticum Fibiger observed extensive benign hyperplasia and hyperkeratosis of the squamous epithelium of the forestomach, consisting in the development of numerous papillomas and warty excrescences and occupying a large portion of the forestomach. He also observed in these rats a histologically different type of hyperplasia which he believed to be carcinoma of the squamous epithelium. Both these lesions he attributed to the presence of Gongylonema. Since benign hyperplasia equal in extent to that found by Fibiger but not associated with carcinoma has since been observed in rats not infected with Gongylonema, this nematode cannot be held responsible for the benign hyperplasia. The factors determining the onset of the benign papillomatosis have not yet been identified. A contributory part may be played by unbalanced diets, especially diets deficient in vitamin A, but they are not the chief determining factor, for, although this extensive papillomatosis was frequently found in rats kept on such diets ten years ago, it failed to appear in experiments carried out recently under apparently identical conditions. Possible explanations for this discrepancy are suggested. The question whether the histologically different type of hyperplasia in the rat's forestomach, which Fibiger believed to be a carcinoma of the squamous epithelium, was true malignant hyperplasia, and if so, whether it was produced by G. neoplasticum, cannot be decided until all the factors responsible for the development of the extensive benign papillomatosis have been identified so that the latter lesion can be made to develop experimentally at will. On the other hand, it is possible to conclude from the experiments of Passey and his collaborators that infection with Gongylonema, even when combined with an unbalanced diet, is not in itself sufficient to produce carcinoma of the forestomach. The general question of the relationship between the presence of benign hyperplasia of the squamous epithelium and subsequent development of malignant growth is briefly discussed. Lastly, the at present erratic development in the rat's forestomach of extensive papillomatosis which may simulate superficially the appearance of malignant new growth must be taken into account in experiments designed to test the carcinogenic action of substances, especially of substances present in food or drink, if grave errors are to be avoided. In this connection it is well to bear in mind that the lesions, when they occur, are restricted to the forestomach, which is lined with squamous epithelium and which has no counterpart in the human stomach.

FROM AUTHOR'S SUMMARY.

LIFE EXPECTANCY AND THE INCIDENCE OF MALIGNANT DISEASE. C. E. WELCH and I. T. NATHANSON, Am. J. Cancer **31**:586 and 598, 1937.

The median length of life in patients with untreated carcinoma of the cervix is fourteen months; of the bladder, fourteen months, and of the prostate, twelve months. The median length of life in patients with treated carcinoma of the vulva is thirty months; of the vagina, twenty-four months; of the cervix, twenty-seven months; of the fundus of the uterus, forty months, and of the ovary, twenty-two months. The life expectancy of young patients with carcinoma of the cervix is less than that of older groups. Adenoacanthoma appears to be intermediate in malignancy between cancer of the cervix and cancer of the fundus of the uterus. The median length of life in patients with treated carcinoma of the penis is seventy-five months; of the testicle, twenty-four months; of the prostate, twenty-six months; of the bladder, twenty-seven months, and of the kidney, twenty-two months.

There appears to be no significant variation in the life expectancy of patients with Hodgkin's disease and those with other types of malignant lymphoma. The median life expectancy for those with Hodgkin's disease is thirty months; of those with the other types, twenty-four months. The median life expectancy of patients with chronic leukemia, either lymphatic or myelogenous, is about forty months. Acute lymphatic leukemia was more common than acute myelogenous leukemia in this series. Treatment prolongs the life of patients with malignant lymphoma about six months. The median life expectancy of patients with fibrosarcoma is forty-three months; with malignant melanoma, thirty-nine months, and with osteogenic sarcoma, twenty-one months. Females with malignant lymphoma appear to have a slightly better prognosis than males, in early years.

FROM THE AUTHORS' SUMMARIES.

PRODUCTION OF BLADDER TUMORS IN DOGS BY BETANAPHTHYLAMINE. W. C. HUEPER, F. H. WILEY and H. D. WOLFE, J. Indust. Hyg. & Toxicol. **20**:46, 1938.

Preneoplastic and neoplastic formations (papilloma, carcinoma) were obtained in 13 of 16 female dogs treated for from twenty to twenty-six months with subcutaneous injections and oral feeding of commercial betanaphthylamine. Periodic cystoscopic examinations and biopsies indicated that the pretumorous changes of the vesical mucosa consisted of the appearance of blanched or red-brown areas and small polypous elevations. The neoplasms were pedunculated and sessile, benign and malignant papillomatous as well as infiltrative carcinomatous growths. Generalized papillomatosis was frequent.

FROM AUTHORS' SUMMARY.

FOWL LEUKOSIS. C. D. LEE and others, J. Infect. Dis. **61**:1, 1937.

The term "fowl leukosis" is used to include the transmissible leukemic, aleukemic and leukemic-like diseases of fowls. Fowl leukosis in all its expressions may be transmitted to healthy chicks by injecting tissue suspensions of affected organs or by injecting cell-free filtrates or it may be transmitted by allowing chicks to come into direct contact within the pen or by rearing them on contaminated litter.

The injection of a suspension made from one type apparently produces all the types considered as expressions of this disease. Different suspensions produced similar results. Neurolymphomatosis associated with ocular lesions, hemocytoblastosis and lymphoid, erythroid and myeloid leukosis are different expressions of the same disease and are caused by a common agent.

The clinical course is variable; in some cases it is comparatively short, and in others it extends over a period of months. No complete recoveries occur. There seems to be a definite inherent difference of susceptibility and resistance between different birds and between different strains. The evidence seems to suggest that the disease may be transmitted through the egg to a limited extent. The most important methods of control are proper sanitation and use of breeding stock from resistant sources.

FROM AUTHORS' SUMMARY.

CARCINOGENIC ACTION OF ESTRONE. G. M. BONSER, L. H. STICKLAND and K. I. CONNAL, J. Path. & Bact. **45**:709, 1937.

The induction of five mammary carcinomas by means of estrone benzoate and estrone combined with prolactin in 32 nonbreeding female mice of a strain apparently refractory to the development of spontaneous tumor of the breast is described. As none occurred in 41 untreated mice it is probable that the treatment determined the development of the tumors. These appeared earlier than spontaneous tumors occurring in breeding females of the same strain in another laboratory. There was no significant difference in action between estrone-benzoate and estrone combined with prolactin as evidenced in yield of tumors or in microscopic appearance of the breast tissue.

FROM AUTHORS' SUMMARY.

CAUSE OF NEW BONE FORMATION IN OSTEOPLASTIC CARCINOSIS OF THE SKELETON. W. SCHMIDT, Beitr. z. path. Anat. u. z. allg. Path. **99**:232, 1937.

In extensive metastatic osteoplastic carcinosis of the skeleton, secondary probably to primary uterine carcinoma, the carcinosis led to marked thickening of almost all the spongy bones investigated as well as to new bone formation in the marrow of the femurs. The external form of the skeleton was preserved with the exception of slight narrowing of a few thoracic vertebrae. The long bones showed areas of atrophy of the blood-forming tissue and new bone formation but no microscopic evidence of carcinoma. Such new bone formation depends, accordingly, not only on a local action of tumor cells but also on endosteal cells. The possibility of new bone formation in connection with local healing of metastases is considered.

R. J. LEBOWICH.

CARCINOMA IN A RENAL FISTULA. H. KUNSTMANN, Zentralbl. f. allg. Path. u. path. Anat. **68**:49, 1937.

Carcinoma originating in the wall of a fistula is uncommon, but occasionally one occurs in the drainage tract of a chronic bone lesion. Less often, carcinoma grows in a gallbladder, colonic or pleural fistula. The renal fistula either is repaired early, reducing the hazard of carcinoma, or is associated with lesions which cause death in a short time. This report deals with a malignant growth which began in the posterior third of a renal fistula of thirty years' standing. The right kidney, ruptured in a fall when the patient was a boy of 7 years, was operated on, with two fistulas resulting in the scar tissue. From these were passed urine, at times soft stones and shortly before death, at 37 years, a large amount of pus. The carcinoma was of a typical highly differentiated squamous variety, with marked keratinization. The pathogenesis of the tumor is considered at length, and it is deemed most probable that the growth began in the squamous epithelium which grew in from the skin and lined the entire tract.

GEORGE J. RUKSTINAT.

MYOBLASTIC MYOMA IN A GRAVID UTERUS. VOSSKÜHLER, Zentralbl. f. allg. Path. u. path. Anat. **68**:116, 1937.

Abrikossoff divided myoblastic tumors into four groups: (*a*) those with cells 20 to 25 microns long and without cross striations; (*b*) those with elongated muscle cells with cross striations; (*c*) those with multinucleated myoblasts from 40 to 60 microns in diameter, and (*d*) myoblastic sarcoma. He originally regarded these tumors as a regenerative response in damaged muscle but later agreed with Linge that they are the result of dysontogenesis. The myoblastic tumor occurs most commonly in the tongue but has been reported in the parotid gland, upper lip, calf, diaphragm, subcutaneous tissue, upper jaw, breast and laparotomy scar tissue. The report of myoblastic tumors in the uterus enlarges the concept of

the distribution of such tumors. Clinically, they are benign; peripherally they are ill defined and merge with the surrounding tissue.

The tumors herein described were pea to walnut size and occurred in a saucer-sized region of a pregnant uterus. The uterus was removed by cesarean section because of atony and prepartum bleeding. The masses were softer than the surrounding uterine muscle and showed small clefts. Histologically, the cells were from 15 to 30 microns in diameter and were round or polygonal. At times, they were elongated, and the homogeneous protoplasm stained weakly with eosin. An internal perimysium was demonstrable with Van Gieson stain. In the largest nodules there was extensive plasmolysis, and the cells were as much as 50 microns wide. The tumors were considered as typical of the "a" group of Abrikossoff.

GEORGE J. RUKSTINAT.

CANCER IN RELATION TO TRAUMA. B. SCHMID, Ztschr. f. Krebsforsch. **45**:113, 1936.

Here are reported 2 cases of cancer, both apparently related to gunshot injuries incurred during the war. The one case was one of pelvic perforation, with necrosis of bone and establishment of a rectal fistula. During the eighteen years following the injury, there was a history of rectal infection, polyposis and eventual cancer. With reference to the two possibilities—first that the neoplastic changes were the result of the inflammatory irritation; second, that a polyposis previously present was irritated by the inflammation to cancerous development—the time relationships speak for the probability of the former. In the second case there was a gunshot wound of the upper angle of the ear, with a subsequent continuous eczematous inflammation. During the following ten or twelve years this became cancerous.

H. E. EGGERS.

QUIESCENT METASTASIS IN NERVES. W. DEPAY, Ztschr. f. Krebsforsch. **45**:127, 1936.

In a patient with cancer of the tongue, who was operated on in 1925, there developed seven years later severe neuralgic pains. When death occurred, two years later, there was found a metastasis in the trigeminal nerve. The article contains a review of the literature on delayed metastasis of cancer.

H. E. EGGERS.

HISTOLOGIC CHANGES FOLLOWING IRRADIATION OF TUMOR TISSUE. R. BAUMANN-SCHENKER, Ztschr. f. Krebsforsch. **45**:178, 1936.

In 3 tumors—a squamous cell carcinoma, an adenocarcinoma of the cecum and a basal cell carcinoma—Baumann-Schenker observed the changes following increasing periods of irradiation. In the first, these took the form of cellular swelling and nuclear degeneration; in the second, there was increased mucous secretion with relatively little necrosis; in the third, marked liquefaction necrosis with especially early disintegration of such cell nests as were present. None of the tumors was suited to observation of the increased differentiation which has been reported by others, but the increased secretory activity shown by the second tumor, Baumann-Schenker believes, is a functional expression of such change.

H. E. EGGERS.

## Medicolegal Pathology

MEDICOLEGAL IMPLICATIONS OF RIGOR MORTIS OF THE NECK. E. H. HANSTEEN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:450, 1937.

Dead bodies were placed flat on a cart in the usual supine position and kept in an ice box before being examined the following morning. The amount of rigor

in the neck was designated as 3 plus when the head could be moved 1 to 3 cm. from front to back, as 2 or 1 plus when the movement was between 3 and 9 cm. and negative when the motion was over 9 cm. Many of these bodies had been moved considerably after death. The author believes that marked to moderate rigor is not influenced by moving the body. This conclusion refutes the findings of Berner, who found that most corpses which he examined were without rigor of the neck after having been moved. The observations of Hansteen may necessitate some revision of the importance of the nonmovement of bodies in medicolegal cases.

GEORGE J. RUKSTINAT.

INJURIES OF THE SPINAL CORD FOLLOWING DIAGNOSTIC PROCEDURES. R. DONAT, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:34, 1937.

In 2 patients the use of lumbar anesthesia was followed after several months by sensory disturbances in the lower extremities and incontinence of urine and feces. Death was due to uremia following ascending urinary infection. The changes in the spinal cord simulated syphilis, although both patients had negative Wassermann reactions. The main alterations involved the leptomeninges and were of a chronic productive and inflammatory nature. The leptomeninges were adherent to the dura dorsally from the middle of the thoracic region caudally. The nerves of the cauda equina were converted into a bundle adherent to the dura and pia. Dorsally, in the regions most severely involved, all three coverings of the cord were thickened and converted into a connective tissue sheath. The picture was typical of chronic pachymeningitis and leptomeningitis with atrophy and necrosis of the spinal nerve roots. The explanation of these untoward effects following lumbar anesthesia is not clear. There is a possibility that the anesthetic was the injurious agent. Therefore, the solution one uses should be fresh. Another possibility is that there was preexisting inflammation of the coverings of the cord. A third is that infection was introduced through a technical error.

A pathologic condition similar to the one described was noted after the use of iodized sesame oil in myelography. The leptomeninges were slightly thickened anteriorly, and yellow-brown streaks of the oil were found in them. Near the collections of iodized oil were areas of granulation tissue. The process increased in severity from above downward. In a chemical investigation of iodized sesame oil and of iodized poppyseed oil, it was found that the stimulant action was not dependent on the iodine contents of these preparations. The oil in which the iodine was dissolved contained unsaturated fatty acids; when the solutions aged, their free fatty acid content increased. The free fatty acids were regarded as the substances causing lesions when injected into the spinal subarachnoid space.

GEORGE J. RUKSTINAT.

CARDIAC INJURIES FROM BLUNT FORCE. W. MUNCK, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:56, 1937.

Thirty-two persons were found to have cardiac injuries after automobile accidents, streetcar collisions, falls from great heights, and a trauma sustained in an explosion. Those with cardiac ruptures were excluded, but otherwise no selection was attempted in this series. Thirteen of the injured persons lived less than one-half hour, while the rest lived as long as sixteen days after their mishaps. The injuries found in the hearts consisted of small hemorrhages, especially in the left posterior papillary muscle. Other extravasations occurred subepicardially or subendocardially but always extended into the muscle. Foci of dilated veins and contracted arteries were regarded as spots of muscle rupture, although this could not be demonstrated definitely because of extensive fragmentation.

GEORGE J. RUKSTINAT.

THE RATE OF GROWTH OF FINGER NAILS. R. WIGAND, Deutsche Ztschr. f. d. ges. gerichtl. Med. **29**:75, 1937.

Wigand, because of his interest in finger nail ridges in cases of nonfatal arsenic poisoning, attempted to establish a normal rate of nail growth. These studies were carried out on nails which had been injured near the lunule. Trauma, however, produced cross ridges of nails, which influenced their growth in some way. In order to acquire standard measurements of nail growth, Wigand etched the nails with fuming nitric acid, producing a stable xanthoprotein reaction. By this method it was proved that on a right-handed person the nails on the right hand grow more quickly; e. g.., 1.2 mm. in ten days as against 1.1 mm. for those on the left hand. The nails of the thumb and small finger grow more slowly than the others.

GEORGE J. RUKSTINAT.

# Society Transactions

## PATHOLOGICAL SOCIETY OF PHILADELPHIA

BAXTER L. CRAWFORD, *President*

*Regular Meeting, March 10, 1938*

ESTIVOAUTUMNAL MALARIA TRANSMITTED THROUGH DRUG ADDICTS: REPORT OF A CASE. J. U. GUNTER.

An epidemic of malignant estivoautumnal malaria of the cerebral type developed among the heroin addicts in the city of New York during 1933 and 1934. Since then the disease has become endemic among that group. A crude syringe consisting of a medicine dropper and a hypodermic needle is used to inject a solution of heroin intravenously. The apparatus is neither washed nor sterilized and sometimes is used by several addicts in rapid succession. Two may even share the same heroin solution in one syringe, and in this manner malarial parasites may be transmitted directly from person to person.

In the present instance a Negro aged 28 years was admitted to the Pennsylvania Hospital (medical service of Dr. Garfield G. Duncan) Dec. 13, 1937, complaining of sore throat, cough and pain in the upper part of the abdomen of several weeks' duration. He stated that he had not had chills, fever or headaches, but the history was unreliable. He appeared acutely ill and was semicomatose. There were twitching movements of the face and arms. The liver was barely palpable. Over the veins of both forearms were pigmented indurated linear scars resulting from intravenous injections.

He had marked secondary anemia and leukocytosis (white cell count, 16,000). Smears revealed malarial parasites in the red cells in great numbers. Signet ring forms only were observed, but crescents were later recognized in bone marrow obtained by puncture. Despite antimalarial therapy, the patient died two days after admission.

At necropsy there was a marked icteric tinge to the conjunctivae. The liver and spleen were enlarged and soft and had a dull slate color. The bone marrow of the ribs and vertebral bodies was grayish black. The other organs including the brain were not remarkable grossly. Microscopically, malarial parasites were exceedingly numerous in the capillaries of the heart, brain and diaphragm and in the splenic pulp; they were found in smaller number in practically every other organ. Granules of black pigment giving the prussian blue reaction for iron were distributed throughout the reticuloendothelial system. The pigment was present in large quantity in the splenic pulp, the bone marrow, the Kupffer cells of the liver, the glomerular tufts and the monocytes of the blood stream.

CHANGES IN THE CENTRAL NERVOUS SYSTEM IN SULFANILAMIDE INTOXICATION. R. P. CUSTER, H. W. FORSTER, W. O. LAMOTTE, D. M. PATTON and J. D. PHINNEY.

During the course of experiments directed primarily toward demonstrating the effect of sulfanilamide on the peripheral blood and blood-forming organs, symptoms were observed suggestive of lesions of the central nervous system, and the present report deals with this phase of the general problem.

Normal dogs receiving by mouth 0.67 Gm. of sulfanilamide per kilogram per day or more showed more or less severe toxic manifestations, which in the milder form consisted of anorexia, vomiting, salivation, apparent vertigo, weakness of the hindquarters, ataxia and a definitely spastic gait. Animals with a more severe

form of the disease exhibited in addition general spastic paralysis with opisthotonos and extensor rigidity of the limbs, increased tendon reflexes, hyperesthesia, apparent dementia and blindness.

During the period of onset of toxic symptoms the animals frequently exhibited circling movements while walking, the initial circling being in one direction, but soon reversing, until the gait became too uncertain for maintenance of an erect posture. During the stage of extensor rigidity the limbs could be forcibly flexed, giving a sensation comparable to bending led pipe; once flexed they remained thus until either the animal or the observer attempted to initiate movement, when the legs immediately snapped into the previous rigid extension. Three of the dogs that were narcotized with pentobarbital sodium during several periods of most intense intoxication showed complete remission of symptoms and immediate induction of sleep, during which the rigidity disappeared entirely and the tendon reflexes returned to normal. All symptoms disappeared spontaneously within from eight to ten hours after the administration of the last dose of sulfanilamide. All animals showed development of marked tolerance to the drug, with wide individual variations.

Grossly, all brains appeared swollen, soft and edematous. Histologically, the edema was apparent throughout, evidenced by the general sponginess, which was particularly marked in the periphery of vessels, where an open zone with frayed margins was observed. Ganglion cells throughout showed degenerative changes which varied in degree from slight swelling to complete cytolysis, the severity varying considerably with the location, being most marked in the hippocampus, basal ganglions and cerebellum. In one instance there was anemic necrosis in the lenticular nucleus. One of the dogs in which fairly chronic intoxication had been established showed increase in the adventitial substance of the small blood vessels.

In the spinal cord and in the peripheral nerves swelling of the myelin sheaths was noted in all animals, partial to complete demyelinization occurring in some of the motor tracts. In the anterior horns of the spinal cord the cells showed degenerative changes similar to those in the ganglion cells of the brain. In one instance the sciatic nerve showed patchy replacement with strands of Schwann cells.

The concentration of sulfanilamide in the blood determined the severity of the neurologic symptoms, that necessary to produce certain initial changes in behavior being essentially the same in the various animals. The correlation between these symptoms and the morphologic changes in the central nervous system is not altogether clear. The symptoms are probably not due to a direct toxic action of the drug but rather to edema. A degeneration of capillary endothelium induced by sulfanilamide is likely, producing increased permeability and consequent edema. The cellular changes are secondary and are similar to those seen where edema and anoxemia are present from whatever cause. The rapid recovery after withdrawal of the drug can be explained on this basis. The effect of the more permanent cellular damage could be demonstrated clinically by exercising a dog which had apparently recovered from a toxic episode and finding rapid fatigability. The possibility of anoxemia playing an essential role is indicated by the reduction by one half in the oxygen capacity of the blood in animals receiving toxic doses; this is probably reflected in the more sensitive portions of the central nervous tissues. Experiments designed to investigate this problem further are being planned.

ARTERIOSCLEROTIC AORTIC ANEURYSM SUCCESSFULLY WIRED: OBSERVATIONS AT AUTOPSY AFTER FOUR YEARS. GEORGE B. CRADDOCK.

A white man of 54 years suffered a contusion of the abdomen in September 1928 and was unable to work for one month. In December 1930 he was found to have a slightly enlarged heart with a systolic mitral murmur and electrocardiographic signs of myocardial disease. He remained in fairly good health, however, until October 1932, when intermittent throbbing abdominal pain developed which

persisted until his admission to the Philadelphia General Hospital in April 1933. At this time a harsh aortic systolic and a softer mitral systolic murmur were noted; the blood pressure was 200 systolic and 100 diastolic. A sausage-shaped abdominal mass with expansible pulsation and a bruit was found. Laparotomy was performed by Dr. Hubley Owen, and a fusiform aneurysm of the abdominal aorta was wired by Dr. Henry Jump. The patient returned to work in October 1933 and continued at work until June 1937, suffering from only slight abdominal pain after the day's work during this period; then cardiac decompensation occurred, from which he never recovered; he died October 6.

Pertinent observations at autopsy (Dr. R. P. Custer) may be taken from the protocol as follows: "The ascending segment and arch of the aorta are slightly dilated and tortuous, the whole vessel being entirely inelastic. The first portion shows only a few elevated yellow intimal plaques which become progressively more numerous, and in the descending thoracic segment, above the diaphragmatic hiatus, the plaques are almost confluent. Here the majority are ulcerated, many being crusted with fibrin and underlaid by a brittle calcific zone. Beginning 2 cm. below the diaphragm, the aorta enters a fusiform mass, 18 by 10 by 6 cm., which extends to the brim of the true pelvis; the ventral surface is formed by a thick tough white membrane, to which the duodenum is intimately adherent. Sharp dissection is required to remove the mass from the vertebral bodies. Section of the aneurysm shows it traversed longitudinally by the original aortic lumen, which is compressed and distorted but appears to be lined by a definite intima; it contains fragments of mural thrombi, which are readily detached. Dense laminated blood coagulum forms a casing, the maximal thickness of which is 5 cm. and the minimal 7 mm. The outer coat is formed by dense fibrous tissue from 2 to 10 mm. thick, which merges with the original adventitia of the aorta; this aneurysm then is a dissection along the medial coat of the vessel, and it still retains communication with the lumen through a slit in the intima immediately below the orifice of the left renal artery, apparently the site of a preexisting ulcerated atheromatous plaque. Regardig the wiring of the aneurysm, the mass of blood coagulum is traversed by strands of gold wire which at three points penetrate the intima and cross the lumen; near one of the strands there is nonocclusive thrombus formation. The fusiform mass extends 4 cm. below the bifurcation, and the thickened, rigid common iliac arteries emerge from it 6 cm. apart. There is no gross evidence of syphilitic aortitis; above the aneurysm the intima is thrown into a few vague longitudinal ridges but hardly in a fashion suggestive of this disease. The peripheral arteries are markedly thickened, rigid and studded with elevated yellow intima plaques and show segmental calcification.

"The heart is markedly enlarged, with a transverse diameter of 15 cm. and a vertical diameter of 12.5 cm. The epicardium shows extensive, irregular opaque white thickening over the entire ventral surface and in patchy distribution over the dorsum. The thickening of ventricular walls is symmetric, the right ventricle measuring 9 mm. in the maximum thickness and the left ventricle 21 mm. The muscle in general is beefy red with prominent narrow gray streaks. In the apex of the left ventricle and extending onto the lower third of the interventricular septum is an area in which the wall is reduced to from 2 to 4 mm. in thickness; at this point the muscle is largely replaced by fibrous tissue, and the endocardium is white and opaque; above this region the left anterior descending branch of the coronary artery is practically occluded. The valves of the right chambers appear normal. The mitral leaflets show fusion, but there is no real stenosis; the leaflets are thickened, especially at the free border, where the chordae tendineae attach directly without the usual delicate webbing. The aortic valve is markedly stenosed, barely admitting the tip of the index finger; there is fusion at the commissure between the right and left cusps, accompanied by marked thickening and heavy deposition of calcium. The cusps proper are only moderately thickened and show patchy calcification; the right cusp presents a small fenestration; there is widen-

ing of the commissures. The coronary orifices are not stenosed; the arteries are thick, rigid and irregularly narrowed, some segments being of pipestem quality."

Other tissues showed the changes of chronic passive congestion. The left ureter was compressed by the aneurysm, producing hydronephrosis. Gangrenous patches on the lower extremities apparently followed dislodgment of the thrombi from within the aneurysm.

Interest in the case lies in the remarkable longevity following the appearance of symptoms caused by the aneurysm. There was apparently no change in the size of the aneurysm after wiring was performed, and so long as an adequate circulatory status was maintained the original aortic lumen was functionally competent.

AORTIC ANEURYSM WITH RUPTURE INTO THE STOMACH. HOWARD MOSER.

A white man aged 72 years was admitted to Jefferson Medical College Hospital, Nov. 14, 1937, complaining of pain in the abdomen, of vomiting blood for two days and of hoarseness for seven months. The abdominal pain was localized in the midline just above the umbilicus. It was constant, dull and severe. On the day of admission, he vomited approximately 2 quarts (about 1,890 cc.) of bloody fluid, and after admission there were several vomiting spells, each time the material being mostly gross blood. Roentgen examination of the chest revealed a large aneurysm in the region of the arch of the aorta. The blood pressure was 98 systolic and 64 diastolic, the temperature 98 F., the pulse rate 68, the respiratory rate 18. The Kahn test was positive. Death occurred three days after the patient's admission, from shock.

At autopsy the first portion of the aorta was dilated and the wall thickened. A large saccular dilatation, which measured 10 cm. in diameter, involved the arch of the aorta. It extended to the left side of the vertebral column and was adherent to the trachea and esophagus. The wall was thin, but laminated clot was present in areas. On the posterior wall, the aneurysmal sac had ruptured, with extensive extravasation of blood into the surrounding tissue and into the esophageal wall, separating the mucosa from the muscular coats and dissecting downward to the stomach, where there was a perforation of the gastric mucosa, approximately 1.5 cm. in diameter. A large clot filled the spaces between the coats of the esophagus. The stomach contained approximately 1.5 liters of partially clotted blood.

ACTION OF ARSPHENAMINES ON SPIROCHAETA PALLIDA IN VITRO. HARRY EAGLE.

It is the general impression that the arsphenamines have no direct killing action on Spirochaeta pallida in vitro. Contrary to this general impression, it has been found that in vitro arsphenamine, neoarsphenamine, silver arsphenamine and arsenoxide (*m*-amino-*p*-hydroxyphenyl arsenoxide) effect a complete immobilization of virulent S. pallida (Nichols strain), obtained from rabbit testicular chancres. Moreover, these immobilized organisms are noninfectious for rabbits, as shown both by testicular inoculation and by subsequent lymph node transfer, and are presumably dead.

The rate at which this antispirochetal action proceeds and the minimal effective concentration of the arsenical depend on numerous variables. Thus, there is a large positive temperature coefficient in the range of from 23 to 37 C. Serum, particles of tissue and in particular a tissue mash inhibit the antispirochetal effect, perhaps because they combine with the arsphenamines. The degree of aerobiasis seems to have little effect.

Under appropriate experimental conditions, arsphenamine and neoarsphenamine have a definite spirocheticidal effect in vitro within eight hours in at least 1:250,000 dilution; and arsenoxide immobilizes the organisms in dilutions of at least 1,000,000. It is of interest that these concentrations are of the same order of magnitude as those attained in vivo after therapeutic administration of these drugs. Experiments are now in progress to ascertain to what extent oxidation products of the arsphenamines, formed under the conditions of the experiment, contribute to their antispirochetal action.

Several implications of possible practical importance may be pointed out. The immobilizing activity of a given arsenical can be assayed by a simple in vitro experiment. It becomes of interest to ascertain the degree of correlation between antispirochetal activity as determined by this in vitro test and therapeutic activity as determined in infected rabbits. Also, the in vitro technic should facilitate the study of the chemical nature of the reaction between arsphenamines and spirochetes and the development of new therapeutic agents with more favorable therapeutic toxic ratios. Work along these several lines of investigation is now in progress.

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## NEW ENGLAND PATHOLOGICAL SOCIETY

FREDERIC PARKER JR., BOSTON, *President*

*April 21, 1938*

J. B. HAZARD, BOSTON, *Secretary*

TWO CASES OF MONOCYTIC LEUKEMIA. LOUISA M. NORTON, Concord, N. H.

There is some divergence of opinion as to the relation of the monocyte to the other cells of the blood. Various views have been held: that monocytes are transitional cells in the development of the granular leukocytes; that they are related to the lymphocytes; that they arise from the reticuloendothelial cells through the histiocytes. Vital stains have helped differentiate monocytes from lymphocytes and from cells of the myelogenous series.

I wish to report 2 cases of acute monocytic leukemia.

CASE 1.—A white woman 48 years old complained of nausea and vomiting, weakness, and difficulty in swallowing of three weeks' duration. Numerous enlarged cervical lymph nodes, discrete and not tender, were present. The spleen was enlarged, the tongue was beefy red, and there were numerous purpuric spots on the skin. The red blood cell count was 2,900,000; the white cell count was 67,400, with 89 per cent monocytes and 2 per cent myelocytes, and the platelet count was 128,000. Blood transfusions were given, and roentgen therapy was carried out over the spleen. On the fourth day the white cell count had dropped to 4,200, with 73 per cent monocytes. Death occurred on the sixth day. Permission for a postmortem examination was not obtained.

CASE 2.—A 10 year old white boy complained of acute abdominal pain of one week's duration. He was pale and acutely ill and presented marked enlargement of the cervical, axillary and epitrochlear lymph nodes, enlargement of the liver and spleen and a brawny swelling of the side of the face. The gums were red, spongy and bleeding. The red blood cell count was 2,100,000; the white cell count was 126,000, with 88 per cent monocytes, of which 2.5 per cent were mature, and the platelet count was 40,000.

Transfusions were given, and the spleen was irradiated but without avail. The patient died on the fourth day after admission. Postmortem examination showed lymph nodes and all organs to be infiltrated with monocytes, the more mature being oxidase positive.

DISCUSSION

FREDERIC PARKER JR., Boston: The monocytic leukemias are of interest from several points of view, especially from that of the basis of diagnosis and from that of their relation to reticuloendotheliosis. I feel there is no relation to the reticulum cell of the lymph node. The cells of monocytic leukemia come from the bone marrow.

In reference to the cases just cited I have two questions: first, as to the identification of the cells in the first case; second, as to the incidence of monocytic leukemia in childhood. In my experience it has been rare.

LOUISA M. NORTON, Concord, N. H.: In the first case the cells were for the most part mature monocytes and oxidase positive.

SIDNEY FARBER, Boston: My associates and I have found it necessary to make the diagnosis of monocytic leukemia in 2 of 55 cases of acute leukemia in childhood.

FREDERIC PARKER JR., Boston: My associates and I first recognized monocytic leukemia in 1928. Dr. R. C. Larabee at that time doubted that it occurred, but two or three years ago, just before his death, he stated that he believed practically all acute leukemia in adults was monocytic.

Supravital staining is of aid in diagnosis. An oxidase-positive cell with an indented nucleus and with no evident maturation of its cytoplasm is to be identified as a monocyte.

SHIELDS WARREN, Boston: What is the reaction to the heterophil agglutination test in this disease?

RALPH B. MILLER, Boston: I tried this test in a case, and it was negative.

TRACY MALLORY, Boston: My associates and I have tried the test in several cases, and in all it was negative. The condition in these cases happened to have been diagnosed at first as infectious mononucleosis.

FREDERIC PARKER JR., Boston: The cells of infectious mononucleosis are of lymph node origin and are oxidase negative. This is a helpful means of differentiating them from monocytes.

FURTHER STUDIES ON CENTRAL AUTOLYSIS OF THE ADRENAL GLANDS. WILLIAM FREEMAN, Worcester, Mass.

Previous studies showed no relation between central autolysis of the adrenal glands and postmortem degeneration in any other part of the body or the length of time from death to autopsy. Neither was there any relation to the antemortem chemical or morphologic alterations in the patient's blood, the patient's age, sex, type of psychosis, length of hospitalization, nutrition or to terminal rectal temperature or to the cause of death. The blood plasma of approximately 70 per cent of the group showed ascorbic acid below the level accepted as the minimum normal. It was further found that a direct relationship exists between the antemortem level of ascorbic acid in the patient's blood plasma and the incidence of central autolysis of the adrenal glands at autopsy.

Another group of patients, dangerously ill and with the level of the blood plasma ascorbic acid markedly below normal, was given intravenous injections of synthetic ascorbic acid in order to raise the level well above the established normal range before they died. Autopsies have been made on 3 of the group thus far, and in not one was any evidence found of central autolysis of the adrenal glands.

DISCUSSION

VALY MENKIN, Boston: Would it not be possible to prove further the correlation you have demonstrated by observing central autolysis of the adrenals in scorbutic guinea pigs?

WILLIAM FREEMAN, Worcester, Mass.: We are planning to carry out such an experiment.

R. A. SLOAN, Boston: I should like to know the basal metabolic rates of some of the patients and also the dosages of vitamin C in the 3 cases last mentioned. What degree of desaturation was present? For instance, it might take a week to saturate a patient with small doses of ascorbic acid if in that patient the acid had previously been at a very low level, or it might be done with only one injection if the level was high.

WILLIAM FREEMAN, Worcester, Mass: Basal metabolic rates of the patients are not known. As to desaturation, the last 3 patients mentioned showed an interesting response to daily intravenous doses of ascorbic acid. The first patient with a beginning level of around 0.35 mg. per hundred cubic centimeters of plasma, showed but moderate, gradual response to the medication, as indicated by daily determinations of the level during fasting, until the level reached around 0.70 mg. per hundred cubic centimeters (which required about a week of therapy) and then there was a geometric rise in the level to correspond with each succeeding dose of the acid. When saturation of the body was complete, it required about 100 mg. of ascorbic acid every other day, given intravenously, to maintain the saturation level. The second patient beginning with the same level as the first patient showed no elevation of this level until after two weeks of intravenous therapy, after which the curve made by daily determinations of the level was similar to that of the first patient. However, the second patient required 100 mg. every day to keep him at a saturation level. The third patient starting with a beginning level of about 0.30 mg. per hundred cubic centimeters of plasma, showed no rise even after a month of daily doses of 100 mg. of ascorbic acid, given intravenously. The daily dose was increased to 200 mg. a day and then to 300 mg. a day, and after about a week the curve of the rise in the ascorbic acid of the plasma was similar to the curves described, but it required 300 mg. a day to keep this patient saturated. All 3 patients showed different responses to a fixed intravenous dose of ascorbic acid, and all showed varying daily requirements of ascorbic acid to maintain saturation.

R. A. SLOAN, Boston: Was infection present?

WILLIAM FREEMAN, Worcester, Mass.: The first patient had chronic ascending pyelonephritis with uremia; the second patient had a gastric ulcer with bleeding, and the third had a carcinoma of the colon with perforation, localized peritonitis and bronchopneumonia.

VALY MENKIN, Boston: Were determinations of ascorbic acid made on the carcinoma?

WILLIAM FREEMAN, Worcester, Mass.: Such a determination was not made.

R. L. GOODALE, Boston: Were there any clinical manifestations of vitamin C deficiency in the patients with low values?

WILLIAM FREEMAN, Worcester, Mass.: None was evident. No bleeding of the gums was found. One of the patients had a positive capillary resistance test.

FREDERIC PARKER JR., Boston: What is the histologic picture in the adrenals showing autolysis?

WILLIAM FREEMAN, Worcester, Mass.: The cells lining the cavities showed slight vacuolation. In the autolyzed portion the cell architecture simply disappeared. The remaining cortical cells showed no apparent change, but studies in this regard are not yet complete.

THE LYMPHOID NODULES OF THE BONE MARROW. ROBERT J. WILLIAMS, Providence, R. I.

This report is based on a study of the literature and of marrows from a series of 265 adults, unselected except that patients with primary diseases of the hemopoietic system were excluded, and from a control series of 23 adults in whom no significant inflammatory lesions were demonstrated in any part of the body. The marrows of 29 per cent of the unselected series and 39 per cent of the control series showed lymphoid nodules varying in size from 550 by 450 microns down to 90 by 90 microns, composed principally of small lymphocytes and frequently showing an "arterial" capillary.

Lymphoid nodules are of frequent occurrence in the bone marrow of man and are associated with active red marrow. They occur in a wide variety of diseases, are frequently found when no significant inflammatory lesion is demonstrated and are present frequently after sudden death due to traumatic injuries. Local lesions in the bone marrow do not account for them. They are found more

frequently in the marrow of persons over 40 years of age than in the marrow of adults under that age. No pathologic significance can be attached to the presence in the marrow of lymphoid nodules of the type, size and number described in this report. The evidence appears to justify the concept that the nodules are essentially normal though perhaps variable constituents of active red marrow in adult man.

DISCUSSION

J. B. HAZARD, Boston: I should like to know on what basis these areas in the bone marrow were identified as lymph nodules. They do not appear to have the configuration of those one is accustomed to seeing in other organs.

ROBERT J. WILLIAMS, Providence, R. I.: These nodules were identified on the basis of two criteria: They occurred as a distinct histologic unit, and the cells constituting them were morphologically identical with those of the follicles of lymph nodes or of the spleen. It is not stated whether or not these lymphoid nodules are true follicles in the sense of those of the lymph nodules or spleen.

FREDERIC PARKER JR., Boston: Were reticulum stains done, and were there mitoses in the centers of the nodules?

ROBERT J. WILLIAMS, Providence, R. I.: No reticulum stains were done. No mitoses were demonstrated in cells identified as lymphocytes.

RALPH B. MILLER, Hanover, N. H.: I should like to ask Dr. Williams if he used the term "lymphoid nodules" purposely instead of "lymphatic nodules."

ROBERT J. WILLIAMS, Providence, R. I.: The term "lymphoid" was purposely used. True lymphatic nodules with definite germinal centers were not seen.

FREDERICK PARKER JR., Boston: What have been Dr. Warren's observations in relation to thyroid disease?

SHIELDS WARREN, Boston: Detailed studies of the marrow with this in mind have not been made. It might be worth while studying marrow sections from this point of view.

B. E. CLARKE, Providence, R. I.: I should like to point out that these studies of Dr. Williams have a definite and practical application. Specimens of bone marrow as material for biopsy are becoming more common. If pathologists are going to venture opinions on such specimens, it is essential that they know what the normal appearances are. Several years ago when I first started getting such material I had a marrow that showed foci of lymphocytes and I made a diagnosis of lymphatic leukemia. Subsequent developments proved that I was right, but I know now, as a result of Dr. Williams' work, that I might just as well have been wrong. Such studies as this, which acquaint general pathologists with the normal, or at least the nonspecific, histologic picture of bone marrow are of fundamental importance.

RALPH B. MILLER, Hanover, N. H.: The term "lymphatic nodules" is defined as referring to a definite aggregation of lymphocytes such as one sees in lymph nodes. The term "lymphoid nodules" is defined as designating lymphatic-like nodules or collections of lymphocytes such as has been demonstrated by Dr. Williams.

FREDERICK PARKER JR., Boston: What percentage of plasma cells was present?

ROBERT J. WILLIAMS, Providence, R. I.: Occasional plasma cells were seen.

ROBERT FIENBERG, Westfield, Mass.: Could there be a correlation with the lymph nodes in the older age groups? Is it a compensation for lymph node atrophy?

ROBERT J. WILLIAMS, Providence, R. I.: The material did not allow one to attempt any such correlation.

FREDERIC PARKER JR., Boston: These appear to be focal accumulations of lymphocytes and not lymph nodules.

## NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

*Regular Monthly Meeting, May 26, 1938*

ROBERT A. MOORE, *Secretary*

A PATHOLOGIC CLASSIFICATION OF ABORTION AND ITS CLINICAL APPLICATION. HENRY C. FALK.

This classification divides abortions into seven groups:

Type 1. In this group the infection is limited to the uterine cavity. The temperature and pathologic process are terminated in fourteen days.

Type 2. In this group the infection has progressed beyond the uterine cavity and into the parametria as evidenced by such conditions as parametritis, perimetritis, perirectal abscess and lumbar abscess.

Type 3. In this group the infection presents itself as thrombophlebitis in the broad ligament.

Type 4. In this group the infection forms a mural abscess. This process is never diagnosed clinically but is found at autopsy.

Type 5. In this group the patient gives a history of abortion and presents the symptoms of septicemia. Pelvic examination usually gives negative results. The blood culture is usually positive, and the patient shows the results of metastatic embolism, e. g., endocarditis or an abscess of the spleen, kidney, lung or brain.

Type 6. In this group the infection is of such virulence that it spreads by direct extension from the uterus through the fallopian tubes into the peritoneal cavity, producing (*a*) gangrenous endometritis, (*b*) gangrenous suppurative salpingitis and (*c*) generalized peritonitis.

Type 7. In this group are the perforations of the uterus with all the associated lesions and processes that may accompany such trauma, e. g., intestinal injury, hemorrhage and peritonitis.

In this grouping an attempt is made to classify the infectious abortions according to their pathologic course.

DISCUSSION

MILTON HELPERN: In discussing Dr. Falk's paper I should like to attest to the fact that every time there was an autopsy by the Office of the Chief Medical Examiner in a case of abortion occurring at the Harlem Hospital the gynecologic staff of that institution came down in full force, and I might say that their presence, with the clinical information which they offered, was helpful and stimulated us to careful observation. Sometimes when one is busy, with many autopsies to do, there may be a tendency not to pay sufficient attention to the minute details of the anatomic pathways of infection, but with the clinician nearby asking questions, perhaps at times annoying but nevertheless pointed, one's observations are made more carefully. We were somewhat acquainted with Dr. Falk's numerical system of classification of cases of abortion. About 50 deaths from septic abortion are reported every year in Manhattan, and all of these are studied with considerable care by the Office of the Chief Medical Examiner. Throughout the entire city there are from 90 to 100 deaths from septic abortion every year that come to the attention of the Office of the Chief Medical Examiner. Many of the subjects are sent to us as victims of frank criminal abortions. Others are sent as having had abortions of undetermined origin, and some are thought from the histories to have had spontaneous abortions, but at autopsy these abortions are rarely found to have been spontaneous. In many instances we find in the uterus definite evidence of traumatism, indicating that induction has been accomplished, or there are other findings which show that the process was not spontaneous. We not infrequently

find perforations of the uterus and occasionally lacerations of the intestines, also fragments of a traumatized fetus in the uterine cavity or in the peritoneal cavity.

As to the various types, we see in many cases extensive endometritis within the cavity of the uterus, with an elevated gray purulent membrane, which usually indicates the original site of the placenta. In these cases the infection can usually be traced into the peritoneal cavity, either by its lymphatic extension through the uterine wall or by its direct extension through the lumen of a fallopian tube, although many think that such extension does not take place, but that the fallopian tubes are involved secondarily. There is no anatomic reason why infection should not take place through the lumen of the fallopian tube, and we as well as others (Martland, H. S.: *Am. J. Surg.* **26**:90, 1934) have seen it occur. Dr. Falk has recognized and designated such direct infection of the peritoneal cavity through the fallopian tube as type 6.

Parametritis when seen at autopsy in cases of abortion designated as type 2 is usually accompanied by generalized peritonitis. The process has usually gone on to invade the peritoneal cavity.

Cases of abortion associated with uterine or ovarian thrombophlebitis and embolic abscesses of the lungs without peritonitis are not as commonly seen as those of the previously mentioned types. Thrombophlebitis without peritonitis and with septic embolism of the lungs can occur without endocarditis. Occasionally, we have seen cases in which there has been direct extension of infection through the uterine wall or through the fallopian tubes, producing peritonitis, and also uterine or ovarian thrombophlebitis with embolic abscesses of the lungs, so that the two types of extension can occur in the same case.

Acute bacterial endocarditis occurs occasionally as a complication of septic abortion. Such cases are confusing clinically when the physical evidence of abortion is not clearcut or when a long period of time has elapsed between the time of induction of the abortion and the death of the patient. In these cases, even though there is a considerable amount of involution of the uterus, one can usually tell by careful gross and microscopic examination that there has been a primary infection in the uterus. The endocarditis is usually of an acute type, and most commonly involves the mitral or the aortic valve, although occasionally it involves the tricuspid or the pulmonic valve. The acute infection is apt to complicate old rheumatic valvulitis and congenital lesions of the heart. We have seen the latter in 2 cases of abortion; in 1 acute endocarditis was superimposed on a defect of the interventricular septum, and in the other, on congenital pulmonic stenosis.

We recently made an autopsy in a case of abortion complicated by tetanus in which the tetanus organisms were cultured from the cavity of the uterus. I am wondering how such cases and also those of gas bacillus infection of the uterus following abortion are classified by Dr. Falk. I suppose they would come under the heading of specific forms of septic endometritis.

PAUL KLEMPERER: Were any bacteriologic studies made in these cases?

ALFRED PLAUT: In the last two decades I have seen very few cases of sepsis after abortion. In previous years I fortunately or unfortunately saw many in a rather large hospital. At that time we also did not consider the direct spread through the lumen of the fallopian tube. We considered the spread in these cases as lymphogenous through the broad ligament and perhaps through the wall of the fallopian tube. I do not know whether reports of histologic examinations are available in the literature to prove or disprove this type of propagation. Years ago, at the Office of the Medical Examiner I saw a case which looked much like one of direct propagation through the lumen of the fallopian tube. I had the same question in mind which Dr. Klemperer has asked: how the different bacteriologic findings, notably staphylococcus, on the one hand, and streptococcus, on the other, were distributed between lymphogenous and hematogenous spread. I have heard thrombophlebitis mentioned, but I have not heard mentioned necrotizing endophlebitis without thrombosis, which I recall having seen frequently in the ovarian vein, notably in streptococcic infections.

HENRY C. FALK: My colleagues and I draw a rather sharp distinction between thrombophlebitis with septic embolism and type 5. A patient with thrombophlebitis will give a typical temperature curve with a negative blood culture. She will have a parametritis—e. g., cellulitis of the broad ligament, with symptoms of an associated infection in the cellular tissues of the broad ligament, for thrombophlebitis as a rule does not occur alone. As a rule it is associated with cellulitis of type 2. Later on, perhaps in the third or fourth week, that patient may have a breaking off of some of these septic thrombi, and they will circulate in the blood as septic emboli, which may lodge in some organ, such as the lung or the spleen. This is an entirely different type of infection from type 5. The patient with an infection of type 5 will have a uterus free from lesions, nothing pathologic in the broad ligaments and a positive blood culture within forty-eight hours after admission.

I wish I could answer the question as to the results of bacteriologic studies and their relation to this classification. We have tried for almost three years to obtain from one of the various groups the services of an expert bacteriologist, to allow us to continue, but without success. We believe that the next advance which will be made in the pathology of the septic and the infectious abortion will be made along bacteriologic lines.

With regard to the direct extension of the infection out through the fallopian tubes, the statement that this has occurred is often questioned. We have sections to prove that it has occurred, sections which show true salpingitis in a tube, and at autopsy in such a case the Medical Examiner can frequently express pus from the fimbriated end of the tube.

EXPERIMENTAL STUDIES OF TISSUE REACTIONS TO LIPIDS: I. THE FORMATION OF ACID-FAST MEMBRANES AROUND CERTAIN OILS AND WAXES. IRVING GRAEF and (by invitation) WILLIAM KAUFMANN and LEO KAPLAN.

Following observations of pulmonary reactions to cod liver oil and mineral oil (Graef, I.: *Am. J. Path.* **11**:862, 1935; ARCH. PATH. **24**:122, 1937), experimental studies were undertaken to elucidate the whole histologic reaction to these and other lipids. Eighteen different lipids were used. The reactions presented here followed transtracheal injection of the following lipids: sperm oil, chaulmoogra oil, cod liver oil, halibut liver oil, tung oil, linseed oil and the ethyl esters of cod liver oil and of linseed oil. Rabbits and monkeys were used, and the injections were made with the animals under light ether anesthesia and in the dorsal position. Observations ranged over periods of from twenty-four hours to three months or, in the experiments with cod liver oil, nine months.

The common finding in the reactions to these lipids was the formation of an acid-fast lipid-containing protein membrane around the oil or the esters. In later stages the membrane was fragmented and phagocytosed by macrophages or foreign body giant cells. Besides this feature there was observed in the early stages of the reaction an acute seropurulent response, with relatively few macrophages. In the posterior and pericentral portions of the lower lobes of the lungs were confluent lesions regularly. Necrosis was observed in the response to tung oil, linseed oil, the ethyl esters of linseed oil and the ethyl esters of cod liver oil. Later abscess formation, sometimes communicating with the bronchial system, was found. With cod liver oil, only slight necrosis of the alveolar epithelium was seen, and no abscess formation was encountered.

After one week there was striking bronchiolar and alveolar epithelial regeneration, but this subsided after from three to four weeks, when the oil became encapsulated. Interstitial fibrosis was variable, being most marked around the abscesses produced by the necrotizing lipids and relatively slight otherwise.

Calcification was not encountered in any of these lesions. Acute pleuritis and obliterating endarteritis were found only in the reaction to the ethyl esters of linseed oil. Thrombosis was observed in the pulmonary arteries in the reactions to ethyl esters of cod liver oil.

Considerable amounts of free extracellular lipid were found up to four weeks, but later the oil became encapsulated in granulomas or was phagocytosed. Little intracellular oil was found in the peribronchial or hilar lymph nodes, and there was no evidence of its being deposited in the reticuloendothelial system elsewhere.

The acid-fast membranes noted around the oil were demonstrated by the Ziehl-Neelsen method. In ordinary hematoxylin-eosin sections they were easily overlooked except with cod liver oil; with this oil they were deeply eosinophilic, sometimes yellowish pink.

Similar membranes were produced in vitro by layering these lipids over serum. They were insoluble in ordinary fat solvents. Because of this finding, sudan IV stains were applied to deparaffinized sections, and the acid-fast membranes dissolved the dye, thus proving the presence of lipid in the membrane. Oil elsewhere was dissolved out by the histologic procedures, as shown by comparison with frozen sections.

In later stages the membranes effectively masked the lipid globules in the macrophages, so that no vacuoles were seen in these cells in paraffin sections. Instead Ziehl-Neelsen and sudan IV stains revealed acid-fast lipid in the cytoplasm of the phagocytes. None was found in polymorphonuclear leukocytes.

The membranes appeared around the oil in the earliest lesions produced by cod liver oil, linseed oil and tung oil or the ethyl esters of the first two. In the lesions produced by chaulmoogra oil, membranes did not form in one week but were observed at four weeks. With sperm oil no membranes were seen after twenty-four hours but appeared in ninety-six hours and increased in amount in longer experiments.

The nature of the membrane is still under study chemically and will be reported later.

DISCUSSION

ALFRED PLAUT: I should like to ask whether Dr. Graef has tried to apply the acid-fast stain and the fat stain to the so-called hyaline membranes one finds in the lungs of newborn infants and in the lungs of adults with different diseases, notably endocarditis. I have never tried it, but after hearing this paper I certainly shall do so.

If one applies the sudan stain to routine paraffin sections, one is astonished how much of the fatty substance is not extracted by the ordinary passage through alcohol and xylene or benzine. The lipofuscins stain almost as well in paraffin sections as they do in frozen sections which have been treated only with 40 or 50 per cent alcohol. The adrenal cortex also retains a good deal of the lipoid substance in the paraffin sections. There is much variety, and I cannot account for it.

N. CHANDLER FOOT: In 1912 Kawamura, a Japanese, described an interesting analytic method for identifying the different lipids tinctorially. He made an extensive use of nile blue sulfate, which is a useful stain. He also employed the Ciaccio stain, which is supposed to be specific for lecithin. I think in this connection it might be interesting to employ some of the stains he used, comparing them with his tables, as possibly indicating the change taking place in these oils during their absorption. I imagine the oils act essentially like neutral fats before they are absorbed, and then, as they are absorbed, are broken up into fatty acids or lipids of various sorts. If this is so, this method of Kawamura ought to be of some use.

IRVING GRAEF: The hyaline membranes in the lungs of the newborn infant and the so-called hyaline membrane in the lungs of the patient with influenzal pneumonia have been tested and found negative to the Ziehl-Neelsen stain. However, I have tested only a few of them, and we are testing more of them right along. Although the former regularly contains lipid (presumably from aspirated vernix caseosa), the possibility exists that some of the lipid is bound to protein and might acquire an acid-fast property in the lung.

With regard to the methods of examination of these lipids, we have used other methods than those referred to. Nile blue sulfate was regularly employed on frozen sections, and it was also found to stain the membranes after paraffinization and deparaffinization of tissue. Of interest was the consistent reaction with the tested lipids, excepting sperm oil and chaulmoogra oil, which have iodine values under 100 and are not as unsaturated as the others. The other oils, e. g., cod liver oil, stain blue with nile blue sulfate, which indicates the presence of what has been interpreted as free fatty acids. I think that interpretation should be qualified—it might represent the reaction to unsaturated fatty acid. With chaulmoogra oil and sperm oil we get the "neutral lipid" reaction, i. e., the reddish pink color, but when these oils are phagocytosed the particles stain blue.

The other method used was polariscopic examination of frozen sections, and this gave uniformly negative results.

DISSEMINATED LUPUS ERYTHEMATOSIS IN A YOUTH, WITH SOME UNUSUAL FINDINGS. ARTHUR GINZLER (by invitation) and T. T. FOX (by invitation).

Acute disseminated lupus erythematosus was first described by Kaposi (1869-1872), and to the original concept important contributions have been made by Libman and Sacks (1924) and by Baehr, Klemperer and Schifrin (1935).

Clinically, the disease is characterized by erythematous cutaneous eruptions, a febrile course of variable duration (sometimes with remissions), a striking predilection for the female sex, blood changes (anemia, leukopenia, thrombocytopenia), evidence of renal damage, and pleuritis or pericarditis. All of these features may not be found in every case. Pathologically, there may be found pleuritis, pericarditis, endocarditis (nonrheumatic, nonbacterial), vascular alterations involving particularly the glomeruli of the kidneys, generalized lymphadenopathy with areas of focal necrosis, and splenomegaly with focal necroses and vascular changes.

The case presented is of interest because of its unusual (though probably not unique) occurrence in a male. It is apparently the first case reported in a male in which have been demonstrated at autopsy the vascular lesions described only in recent years that are so characteristic of the disease. It is also of interest because of the extent of these and other lesions.

The subject, a 17 year old boy, became ill with the condition in question Oct. 12, 1937, and died March 3, 1938. The course of the disease was characterized by the successive development of polyarthralgias, fever, marked muscle tenderness and wasting, and, shortly before admission to the hospital, a butterfly erythematous eruption on the face and lesions of the finger tips, knees and elbows. On admission to the hospital (Jan. 1, 1938) he presented the typical cutaneous manifestations of acute disseminated lupus erythematosus. In addition there were leukopenia, anemia, urinary evidence of renal damage, changes in the electrocardiogram, serologic tests moderately positive for syphilis (considered nonspecific) and evidence of marked muscle tenderness and atrophy with severe contractures of joints. He pursued a progressively downhill, febrile course. During his stay moderate generalized lymphadenopathy developed; biopsy showed necrotic foci in a lymph node. Clinical and electrocardiographic evidence of pericarditis appeared. Blood cultures, tuberculin and Frei tests and serum agglutination tests for typhoid, paratyphoid A and B., undulant fever and tularemia were negative. The agglutination titer of the serum for hemolytic streptococci was 1:640. Later thrombopenia developed. The blood pressure, at first normal, became elevated. Petechiae appeared on the finger tips. Terminally the boy had a number of convulsive seizures, and he died, with evidence of bronchopneumonia, a little less than five months after the onset of the first symptoms.

Autopsy, performed six hours after death, showed bronchopneumonia, universal adhesive pericarditis, marked cardiac hypertrophy, recent infarction of the spleen and right kidney, generalized lymphadenopathy with focal necroses, and splenomegaly with focal necroses. Microscopic studies revealed severe damage to the glomeruli with hyaline-like thickening of the capillary basement membrane, typical "wire loop" lesions and proliferative and marked degenerative changes of the

endothelial and epithelial cells of the glomerular tufts. The lymph nodes showed numerous areas of focal necrosis containing material staining deeply with hematoxylin. The spleen showed numerous areas of periarterial necrosis in addition to changes of the vessels. Within the tricuspid leaflet of the heart was a single inflammatory lesion; the heart also showed organizing pericarditis. The muscles showed marked atrophy, irregular degenerative changes and occasional mild inflammatory infiltrations. Throughout the connective tissues of the body there were distinct thickening and dilatation of the capillaries. No tubercle bacilli or other organisms were demonstrated in any of the lesions.

This case, as well as a review of the literature, suggests that tuberculosis plays no role in the etiology of the disease. No definite evidence is found pointing to any specific causative factor. Until further information is available, we must conclude that the cause is a toxic agent, not necessarily a specific one, which, perhaps because of constitutional factors in the affected person, produces widespread degenerative and necrotic lesions of the mesenchymal tissues.

DISCUSSION

E. LIBMAN: I cannot go over all the points that have been covered in this splendid presentation.

Of the 4 patients with a like condition which Dr. Sacks and I described, 2 had erythematous eruptions and 2 did not. There was some confusion at the beginning because the dermatologist of the hospital at the time said that while the eruptions closely resembled lupus erythematosus he would not be willing to classify them definitely as such. Shortly after we published these cases a fifth patient was encountered who had a secondary infection with nonhemolytic streptococci, the heart showing so-called atypical verrucous endocarditis and also subacute streptococcic endocarditis. In this case our dermatologist was again unwilling to classify the eruption definitely as lupus erythematosus, but three other dermatologists at the Post-Graduate Medical School and Hospital made that diagnosis definitely. We believe that today the diagnosis of lupus erythematosus would be made in all these cases. In 1925 I ventured the opinion that the disease under consideration could occur without endocarditis. When we first described the disease, there was no definite designation for it, and we named it after the striking form of the associated endocarditis. We believed that the clinical picture was unique.

From our knowledge of the clinical picture, from the histologic studies made by Dr. Louis Gross (not all published) and from a consideration of the vascular lesions that have been described, it is evident that we are dealing with a generalized disease which may be accompanied by a macroscopic peculiar form of endocarditis or by lupus erythematosus or by both endocarditis and eruption or by neither. For the diagnosis in the cases without the macroscopic endocarditis or the eruption it would be necessary to search for the histologic changes found by Dr. Gross and the vascular lesions that have been described. The so-called wire loop lesion is not found in all cases but is characteristic.

Dr. Gross made an elaborate histologic study of the endocardium and pericardium and found characteristic lesions in at least three quarters of the cases. Besides the lesions that he considered definitely diagnostic he found other lesions which are helpful in recognizing the disease. For example, in the next to the last picture shown by Dr. Ginzler there is demonstrated an active lesion in the valve but no lesion at the ring. That excludes rheumatic endocarditis. Such a lesion does not correspond to anything except that found in the disease under consideration.

We are indebted to Dr. Ginzler for his careful studies of the necrotic lesions in the lymph nodes and spleen. In the full description of the disease that Dr. Sacks and I gave, we drew attention to the occurrence of petechiae, especially those with light centers. It has been thought that they could not be of much value for diagnosis because of confusion introduced by the widespread cutaneous lesions. To make use of the petechiae for diagnosis, one must consider only those not located near the erythematosus lesions of the disease.

In this disease there may be found a combination of fresh fibrinous pericarditis and white-centered petechiae. This also occurs in the occasional cases in which there is a combination of subacute bacterial endocarditis and active rheumatic endocarditis, but in the latter kind of case nonhemolytic streptococci are usually found in the blood.

Dr. Sacks and I referred to the subject of the Osler syndrome—visceral lesions of the erythema group. An occasional writer thought that we were identifying the condition in our patients with that syndrome. Of course, that is not correct. Osler realized that he was describing a picture that could be due to various causes. In fact, case 26 of the series of Osler was picked by us as probably being of the same type as our cases.

PAUL KLEMPERER: The lesions which Dr. Ginzler has shown, especially the renal lesions, are really typical, and this is, I think, an important contribution. The 1 male patient reported in our series did not show any lesions. We included his condition among our 23 observations because we accepted every case diagnosed as one of lupus erythematosus clinically, although we were not certain that this case was an actual instance of disseminated lupus erythematosus. The patient who was the subject of observation by Dr. Ginzler is then the first male in whom lesions have been found identical with those classically present in the female.

I have always felt that one should not emphasize too much the importance of the anatomic lesions in this disease. I have always felt, and I think Dr. Denzer has expressed it clearly in his paper, that these lesions are more or less only manifestations of something more general; they are not the disease. Lupus erythematosus is not merely a vascular disease; it is not only a cardiac disease. I think one cannot call it even a disease of the mesenchyme, although the mesenchyme shows definite involvement. We had a case in which there was a peculiar mediastinitis and another case in which there was a definite inflammation around the adrenals and of the retroperitoneal tissue, but all such anatomic lesions are nothing but an index. One can make the diagnosis with such lesions present, or at least seriously consider it.

In this respect Dr. Libman has referred to the cardiac lesions. I will refer to the renal lesions. When they are present, I suspect that the condition is lupus erythematosus. In eclampsia similar lesions are present, but they are much milder. My colleagues and I had 3 cases, however, in which no morphologic lesions were present. In these cases a very acute process had run its course in a few weeks, and we did not find in these cases any lesions even on close histologic examination. It is a well known fact that the macroscopic examination of the organs is very unsatisfactory in this disease. Lupus erythematosus disseminatus is not a disease which can be localized in any organ. Organic lesions, if found, are just an index of something, and one does not know what that something is. I think the only way to find out is to try to reproduce these lesions experimentally. That we have not been able to do, and we have alluded only once to the fact that toxins in general may produce vascular lesions, as Apitz and Gerber have shown. However, the vascular lesions which are produced experimentally are not identical with the lesions found in lupus erythematosus, but there is this one common denominator: their location in the vasculature.

E. LIBMAN: Additional evidence that this is a generalized disease in which the cutaneous manifestations may or may not occur is furnished by the patients who have the general symptoms for months before the eruption develops. For example, recently a patient in Brooklyn came under my observation who was sick for a number of months and then, only one week before death, presented small facial lesions. There was no evidence of endocarditis. At postmortem examination typical lesions were present in the endocardium and the kidneys.

Involvement of joints is frequent. In this connection I should like to draw attention to one of the groups of cases of nonbacterial thrombotic endocarditis,

described by Gross and Friedberg. In this group deformities of joints are present and polyserositis. It is probable that further study will show that some of these cases represent the disease under discussion.

I consider it important to emphasize what Dr. Klemperer has just said, that the idea that the disease is primarily a vascular one is incorrect. There are cases in which there are no vascular lesions, others in which vascular lesions are confined to the kidneys and some in which the vascular involvement is widespread. I might add that occasionally the vascular lesions are those of periarteritis nodosa.

ROBERT A. MOORE: I have nothing to contribute on the pathologic character of disseminated lupus erythematosus, nor any comment to make on this most excellent paper, but I should like to say something concerning a case of disseminated lupus in a 29 year old woman which recently came to our attention at the New York Hospital. The autopsy was made by Dr. Jacob Furth. There are several points of interest in connection with the discussion. First, in the case observed at the New York Hospital migratory polyarthritis initiated the disease and involved the knees, shoulders, wrists and hands. The total duration was approximately sixteen months. Second, the skin lesions were brought on, as is so frequently reported, by a severe sunburn. Third, the renal lesions were not such as have been described. The typical change in the glomeruli and in the basement membrane was not seen, but rather my associates and I made a diagnosis of chronic pyelonephritis. Fourth, there were serofibrinous pericarditis and bilateral pleurisy. Fifth, there were no lesions of the endocardium. Sixth and finally, a point of the greatest importance in our case, there was undoubted tuberculosis in the periportal and periaortic lymph nodes. There were also clusters of fibrocaseous tubercles in the upper lobe of the left lung and caseous tuberculosis of the tracheobronchial lymph nodes.

ARTHUR GINZLER: I have little to add to the discussion except on a few other specific points. The single inflammatory valvular lesion in no way resembles the lesions in rheumatic fever, and in the absence of ring lesions or the finding of any Aschoff bodies in the heart there would certainly be no confusion with rheumatic fever.

Concerning the joints, it was our impression that the contractures which were present in this case were probably due to the extreme muscle tenderness and sensitivity of which this patient complained. Histologic examination of the synovial tissue and synovial lining from the knee joint, which was one of the last joints involved, showed no histologic alterations. In this case also we made an attempt to elicit any history of unusual illness after sunburn or other evidence of photosensitivity, and we were unable to do so. There is no doubt that progressive tuberculosis may sometimes be coincidently associated with this disease, as might be expected in such a debilitating illness.

# Book Reviews

**The Patient and the Weather.** By William F. Petersen, M.D., with the assistance of Margaret E. Milliken, M.S. Volume IV, part 2. Organic Disease: Hypo and Hyperthyroidism, Diabetes, Blood Dyscrasias, Tuberculosis. Pp. xxviii and 729, with 380 illustrations. Price $11. Ann Arbor, Mich.: Edwards Brothers, Inc., 1937.

Part 2 of volume 4 is of a stature to warrant being a volume in its own right. In it Dr. Petersen discusses hypothyroidism, hyperthyroidism, diabetes, the blood dyscrasias and tuberculosis in their relations to the meteorologic environment. He "endeavors to present disease (i. e., the reaction of the human to an unfavorable environmental situation) in the stage of fluid transition from dysfunction to the anatomically defined types with which textbooks deal." He does this, as in previous volumes, by means of numerous case studies illustrated with many graphic charts.

The thyroid dysfunctions and the blood dyscrasias lend themselves well to Dr. Petersen's thesis. From the labile state without increased basal metabolism and without detectable organic lesions, which some have termed autonomic imbalance, to true exophthalmic goiter with increased basal metabolism and with anatomic changes in the thyroid, the heart and the lymphoid apparatus is not a far cry. The reactions to the environment are extreme and violent, to a degree which might be termed illogical and certainly to an extent which falls within the field of the abnormal. If the normal person, whose reaction pendulum swings within a narrow arc and within normal limits, is a cosmic resonator, the person with hyperthyroidism who has violent swings beyond the limits of the normal, might be termed, in the extravagant vocabulary of Hollywood, a supercosmic hyperresonator. Whether the pyknic type exhibits lessened thyroid activity and the leptosome type increased thyroid activity because the one is pyknic and the other leptosome (p. 65), or whether one is pyknic and the other leptosome because of lessened or increased thyroid activity might make a nice subject for a hair-splitting debate. The fact remains that one is hypoactive, the other hyperactive, to his environment.

That reaction to the environment may lead in one person to one type of blood dyscrasia and in another person to a dyscrasia of diametrically opposite type and in a third person at different times to both types of hemopoietic tissue response is an appealing thought which might make molehills out of a number of hematologic mountains. The one, and at this late date it seems to be the only, bit of knowledge that this reviewer carried out of his course in pharmacology, under one of the masters of this science, was that everything first stimulates and then depresses. Granted the fundamental truth of this lore, and it seems to be axiomatic, one has no difficulty in believing that what may stimulate the bone marrow in one person, producing a blood picture that one terms polycythemia or leukemia, may in another depress the marrow and lead to anemia or granulocytopenia.

In diabetes mellitus, too, the clinical manifestations of the disease vary with the constitutional habitus. But the latter, whether pyknic, normosome or leptosome, is associated with variations in the degree and character of the reactions to the environment and may indeed, as the author has previously maintained, be the result of such variations impressed on the subject through his ancestry. For Dr. Petersen diabetes "is primarily a dysfunction and may, indeed probably does, exist with no obvious anatomical alterations." In the pyknic type, whose tissue inadequacy develops slowly because of his lessened reactivity, diabetes develops late in life and runs a relatively benign course. In the leptosome, with his stormier

reactivity, the disease may develop early and is clinically more severe. Diabetes "can be influenced by . . . any of the innumerable environmental factors that find their reflection in the autonomic apparatus. It is closely linked with vascular insufficiency."

Tuberculosis is not so much an immune reaction as a "physico-chemical-enzymatic phenomenon," in which it is of greatest importance "whether the digestive, the catabolic factor, overbalances the anabolic, inhibitory . . . forces." The author associates "clinical recovery with lessened digestion; clinical activity with increased digestion." "The outcome of the disease is meteorologically conditioned. Death or recovery lie not in the lap of the Gods but depend in part at least on the fitfull mood of Asclepius."

Typographic errors are fewer than one would expect in a mechanical process making use of a typewritten master stencil. To the large volume of the written matter are probably to be ascribed the occasional grammatical errors that exist (e. g., the plural "lie" after "Death or recovery," to say nothing of the double "l" in fitful, of the passage just cited, or the sentence on p. 9 "if any one . . . are"). The Tindall of p. xii is presumably Tyndall (1820-1895), the physicist-philosopher friend of Huxley, and not the Tindal (1653-1733) who wrote polemics on religion.

**Review of Textbook of Clinical Pathology.** By Roy R. Kracke. Pages 554. Price $6. Baltimore: William Wood & Company, 1938.

Dr. Roy R. Kracke has edited, and written parts of, a very complete textbook of clinical pathology. The contributors to the respective chapters and sections are experienced workers in their fields, and each has chosen a favorite subject for elucidation.

Twenty-seven chapters take up in systematic order the preparation of reagents, solutions, glassware and other apparatus needed. Then follow chapters on blood, including the preparation of smears, the counting of blood elements, the determination of hemoglobin, the measurement of corpuscular volume and other necessary examinations. Diagnostic agglutination tests are described; then tests of the urine, of the feces, of sputum, of gastric contents, of cerebrospinal fluid and of transudates and exudates and various miscellaneous tests. In the last chapter the tests are grouped in such order that specific diseases are emphasized with the corresponding diagnostic procedures, such as the various anemias, hemorrhagic diseases, disorders of liver function and of renal function, and syphilis.

In all of the chapters there are short résumés of historical interest showing the development of the various tests, followed by short high lights on the physiology of the various subjects. The significances of findings are indicated. The styles of the various authors are somewhat similar. The sentences are short, crisp and concise, making for easy reading and quick reference. Details of the technic are given in lucid completeness. The text is amply illustrated with good pictures showing the methods of preparation of specimens, their collection and their examination.

The book contains in its 554 pages a remarkably large collection of material. It can be kept profitably on the desk of the clinical pathologist for rapid reference and even on the laboratory table, because sufficient details are given to carry out from start to finish any of the ordinary examinations needed plus a considerable number of those not in ordinary use. For all of the materials in this book the index seems adequate, although occupying comparatively few pages.

The book, therefore, can be recommended highly to the general practitioner looking for laboratory help in his diagnostic problems, to the clinical pathologist as a quick and ready reference and to the technician actually working in the laboratory who wants to find concise and accurate directions for the performance of any particular test, starting with the collection of specimens and carrying right through until the test is completed.

# Books Received

SULFANILAMIDE THERAPY OF BACTERIAL INFECTIONS. Ralph R. Mellon, M.D., Dr. P.H., D.Sc. (Hon.), Director, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh; Paul Gross, M.D., Pathologist to the Institute, and Frank B. Cooper, M.S., Research Chemist to the Institute. Cloth. Pp. 386, with 16 illustrations. Price $4. Springfield, Ill.: Charles C. Thomas, Publisher, 1938.

A TEXTBOOK OF BACTERIOLOGY. Edwin O. Jordan, Ph.D., Late Andrew McLeish, Distinguished Service Professor of Bacteriology in the University of Chicago; Revised by William Burrows, Ph.D., Assistant Professor of Bacteriology, University of Chicago. Twelfth edition. Cloth. Pp. 808, with 197 illustrations. Price $6. Philadelphia: W. B. Saunders Company, 1938.

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY. W. A. Newman Dorland, A.M., M.D., F.A.C.S., Lieut.-Colonel, M.R.C., U. S. Army; Eighteenth edition, revised and enlarged. Flexible and stiff binding. Pp. 1607, with 942 illustrations. Price: plain, $7; thumb indexed, $7.50. Philadelphia: W. B. Saunders Company, 1938.

A TEXTBOOK OF HISTOLOGY. Alexander A. Maximow, Late Professor of Anatomy, University of Chicago, and William Bloom, Associate Professor of Anatomy, University of Chicago. Third edition. Cloth. Pp. 668, with 542 illustrations. Price $7. Philadelphia: W. B. Saunders Company, 1938.

A TEXTBOOK OF BACTERIOLOGY. Thurman B. Rice, A.M., M.D., Professor of Bacteriology and Public Health at the Indiana University School of Medicine. Second edition, revised. Cloth. Pp. 563, with 121 illustrations. Price $5. Philadelphia: W. B. Saunders Company, 1938.

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# CONTENTS

PAGE

Lesions of Hypoglycemia: II. Some [illegible] (A. P. [illegible], M.D.), Minneapolis ..... 765

[illegible] Distribution of Tumors in the [illegible] of the Rats (Arthur Weil, M.D., Chicago ..... 777

[illegible] Infant Brain. C. R. [illegible], M.D., [illegible] Island, N. Y. ..... 791

[illegible] of the Skin of Mice Due [illegible] plica- [illegible] of Carcinogenic Agents. Robert [illegible], M.D., [illegible] ..... 800

[illegible] of Spontaneous [illegible] Car- [illegible]oma in Inbred Mice of the A Strain. [illegible] C. [illegible], Ph.D., New Haven, Conn. ..... 814

[illegible] and Other Lesions in Calves [illegible]s Low- [illegible]ington. L. A. Moore, M.S.; E. [illegible]lman, V.M., and L. H. Sholl, D.V.M., East [illegible], Mich. 830

[illegible]-with-Without Syphilitic Lesions [illegible]ttered [illegible] Postmortem Examinations. E. [illegible], M.D., [illegible]polis ..... 839

[illegible] Adenomas of the Adrenal. Mar[illegible] Barker, [illegible], Akron, Ohio ..... 843

Duodenal Ulcella: Variations [illegible]ologic [illegible] Lesions of the Mucosa. [illegible]ie H. [illegible], Rochester, Minn. ..... 853

[illegible] Lymphangiectasia of the Adre[illegible] Milton [illegible]son, M.D., New York, and Edward [illegible]eman, [illegible], M.D., Wichita, Kan. ..... 869

[illegible] Essential Xanthomatosis (T[illegible]-Mann- [illegible]) Associated with Primary Carcinoma [illegible] Liver [illegible] an Infant. Arnold Wood, M.D., Bost[illegible] ..... 873

Laboratory Methods and Technical Notes: [illegible] Fixation Mixtures for Handling Tissue Sec[illegible] Frozen, [illegible] Paraffin and Pyroxylin). George W. L[illegible], A.B., [illegible] York ..... 882

[illegible] and Neurol[illegible] ..... 884

Abstracts from Current Literature ..... 885

[illegible] Society of Philadelphia ..... 904

[illegible] Pathological Society ..... 908

[illegible] Pathological Society ..... 912

[illegible] ..... 920

Archives ..... 922